ENDEAVOUR

A quarterly review, published in five languages,
designed to record the progress of the sciences
in the service of mankind

VOLUME XX April 1961 NUMBER 78

CONTENTS

061.22(43):5:070.432
Editorial: Jubilee of the *Max-Planck-Gesellschaft* 59

541.71
Electron-deficient Compounds — F. G. A. STONE, M.A., Ph.D. — 61

551.51:629.19:525
Space Research — SIR HARRIE MASSEY, B.A., M.Sc., Ph.D., F.R.S. — 68

576.8.097.5:576.088.5:543.426
Fluorescent Protein Tracing and the Fluorescent Antibody Method — R. C. NAIRN, M.D., Ph.D. — 78

533.5:621.52
Ultra-high Vacuum — G. LEWIN, Ph.D. — 85

547.78:577.18:615.779
New Penicillins, Cephalosporin C, and Penicillinase — E. P. ABRAHAM, M.A., D.Phil., F.R.S., and G. G. F. NEWTON, M.C., M.A., D.Phil. — 92

591.185.1:599.4
Echolocation by Bats — J. D. PYE, B.Sc. — 101

049.3
Book Reviews 111

Short Notices of Books 115

Notes on Contributors 116

Some Books Received .. .. Cover iii

Editor: TREVOR I. WILLIAMS, M.A., B.Sc., D.Phil., F.R.I.C.
Assistant Editor: G. N. J. BECK, B.Sc., A.M.I.E.E.
Editorial Assistant: W. A. OSMAN, B.Sc., M.Sc., A.R.C.S.

Imperial Chemical Industries Limited, Millbank, London, S.W.1
Communications to the Editor should be addressed to North Block, Thames House, Millbank, London, S.W.1

Jubilee of the *Max-Planck-Gesellschaft*

RECEIVE
17 APR 196
LIBRARY
WIDNES LABORATORY
WIDNES

This year marks an important anniversary in German science, the jubilee of the foundation of the *Kaiser Wilhelm-Gesellschaft zur Förderung der Wissenschaften.* Since the foundation of this Institution was in itself directly linked with the celebration of the centenary of the University of Berlin, the event is a doubly significant one in the history of science in Germany.

The foundation of Berlin University was a consequence of earlier political developments. When Napoleon created the Kingdom of Westphalia for his brother Jerome in 1807, Prussia lost the famous University of Halle. To replace it, Frederick William III set up in 1810 a new university to provide a centre for learning in his capital. Today this university lies in the East Sector of Berlin, and has been renamed the Humboldt University after Karl Wilhelm von Humboldt. The Free University of Berlin was established in 1948 to serve the needs of the West Sector.

Shortly before the celebration of the centenary of Berlin University, Adolf von Harnack, the great theologian, prepared for the Kaiser a concise but forcefully worded memorandum stating the case for the establishment in Germany of centres for pure research. Although Humboldt himself had stressed the need for original work a century earlier, the emphasis in the German universities at that time was on teaching rather than research. Van't Hoff, who had come to Berlin from Amsterdam in 1896, was almost unique in holding a professorship that entailed no teaching duties. Von Harnack's memorandum had the effect desired, for in the course of the Berlin University centenary celebrations the Kaiser announced a scheme for the setting up of the group of research institutes that in due course became known as the *Kaiser Wilhelm-Gesellschaft.* The necessary endowment was raised by public subscription and Adolf von Harnack became the first President. After the first world war, support from the public and from industry proved insufficient, and the Institution was increasingly supported by the state.

On von Harnack's death in 1930 the Presidency was assumed by Max Planck, who, after an already brilliant career that included the award of a Nobel Prize in 1918, had joined the board of management of the physico-chemical laboratory in 1926; that it was able to attract so great a scientist is a measure of the reputation that the *Kaiser Wilhelm-Gesellschaft* had established in a surprisingly short time. He became the head of a research organization of very wide ramifications, carrying out work in all the principal fields of chemistry, biology, and medicine; in all there were by then some thirty separate research institutes in various parts of Germany, with a few situated in other countries. In Berlin, for example, were the institutes for chemistry; for physical chemistry and electrochemistry; and for experimental medicine. Research relating to iron and steel was carried out at Düsseldorf, and on problems relating to coal and its utilization at Mülheim. Outside Germany were a marine biological research laboratory in Italy and a microbiological laboratory in Brazil.

One of the aims of the *Kaiser Wilhelm-Gesellschaft* was the creation of an institute devoted to pure physics, as distinct from applied physics, which was already the province of the *Physikalisch-Technische Reichsanstalt*—a model for Britain's National Physical Laboratory of 1900—that had been established at Charlottenburg in 1888, with the great von Helmholtz as its first director. Although Einstein was proposed for, and accepted, the post of director, the physics institute was not in fact built until the 1930s, owing to the first world war and the difficulties that followed it. As long as the institute had no building of its own, its activities were, in the main, restricted to the award of research fellowships tenable at other seats of learning. In the desire to attract men of the very highest calibre, special inducements were offered to Einstein to persuade him to return to Germany from Switzerland. He was made a member of the *Preussische Akademie der Wissenschaften,* and given a professorship in Berlin University that left him free to pursue his research. Further, he was allowed the exceptional privilege of retaining his Swiss nationality.

Commemorative volumes published when the *Kaiser Wilhelm-Gesellschaft* celebrated its twenty-fifth anniversary in 1936 remind us that associated with it had been a remarkably large number of men of the highest international repute in science and technology, of whom only a few can be mentioned here. At the physico-chemical and electrochemical laboratory, for example—situated in what was named, as a courteous gesture to British science, the *Faradayweg*—the board of management included not only Planck, but Carl Bosch, a Nobel Laureate of 1931. The name of Bosch will always be remembered with that of Fritz Haber, awarded a Nobel Prize for chemistry in 1918, for the Haber-Bosch process of ammonia synthesis has long been, and shows every sign of remaining, one of the most important of all industrial chemical processes. Haber, who died in 1934, had been associated with this institute from its earliest days, having been appointed its first director in 1911. At the Institute for Chemistry was Otto Hahn, whose Nobel Prize was to be awarded in 1944; closely associated with Hahn was Lise Meitner. Yet another Nobel Laureate, Otto Warburg, was at the Institute for Cytophysiology. The names of Gustav Krupp, Karl von Siemens, Albert Vögler, and Fritz Thyssen on the board of management that controlled the Institution are reminders of the great interest of German industrialists in the project.

Unhappily, however, the twenty-fifth anniversary celebrations corresponded with a serious decline in the fortunes of the *Kaiser Wilhelm-Gesellschaft.* 1933 had seen the National Socialists swept into power, with all the dire consequences that this event entailed for Germany and the world. The violent anti-semitism of the new régime was tempered by neither sense nor humanity; not even such men as Einstein were spared, despite the exceptional concessions that had been made to induce him to return to Germany in the first instance. When the persecution began, those Jewish scientists fortunate enough to escape with their lives found refuge in other countries, where many were able to resume their researches. Germany's loss was the world's gain.

This unhappy period, culminating in six years of war, is one best forgotten in the present context. Its end found the *Kaiser Wilhelm-Gesellschaft*, in common with virtually all other German scientific institutions, utterly disorganized and the staffs of the individual institutes widely scattered. Very early in the allied occupation, the British authorities gave much thought to how academic science in Western Germany could best be revived, and inevitably the future of the *Kaiser Wilhelm-Gesellschaft* received particular attention. Immediate steps were taken—through a scientific advisory council in Göttingen, composed of leading professors from the university there—to re-establish contact between individual laboratories of the Institution in the British and, where possible, other zones. In the new circumstances a change of name seemed desirable, and after careful deliberation it was decided to commemorate the life and work of Max Planck. The British occupation authorities were the first formally to recognise the *Max-Planck-Gesellschaft*, with Otto Hahn as its President; a year was to pass before the American and French governments followed suit.

The change of name, marking the beginning of a new era, was a most appropriate one. Max Planck, President of the Institution in happier days was internationally held in respect and admiration for both his scientific genius and his personal qualities. The quantum theory which he formulated at the beginning of this century can fairly be said to dominate the whole field of modern theoretical physics, and it is unnecessary in these pages to enlarge upon its importance. In 1946 Planck came as an honoured guest to England; although great age had brought physical infirmity, his mind was still extraordinarily clear. The occasion was a particularly appropriate one, for it was the Royal Society's international celebrations to mark the tercentenary of Newton's birth. Newton, it will be recalled, ascribed to light certain wave properties, yet it seemed to him that in some respects the corpuscular theory was the more satisfactory. Planck's work both emphasized the dual nature of radiation and paved the way to later research that finally, through wave mechanics, resolved the paradox and established a unity of wave and particle.

Today, the *Max-Planck-Gesellschaft* stands firmly established. In its more than forty institutes important contributions are being made to biology, chemistry, medicine, and physics. In addition, there are three institutes for the study of law and one for economics; in Rome is the Hertz Library, now the Institution's only establishment outside Western Germany. Some of these laboratories represent the rebuilding of ones damaged or destroyed during the war, others are entirely new. Although financial support comes mainly from the state governments, with some industrial and private help, the independence of action that was so important a feature of the early years of the *Kaiser Wilhelm-Gesellschaft* has been regained. There are firm links with the universities, and several directors of institutes are also university professors. The high standard of the early years seems to have been fully regained, for the staff again includes several Nobel Laureates.

This regaining of its former lustre is typical of German science generally. ENDEAVOUR is happy to send congratulations and good wishes for the future to the *Max-Planck-Gesellschaft* on this important anniversary in its history.

Electron-deficient compounds

F. G. A. STONE

A fairly large group of covalent compounds is known that does not conform with the usual rules relating chemical composition with classical valence theory. These substances, in which there appear to be fewer valence electron-pairs than there are chemical bonds, have been described by the term 'electron-deficient'. This article describes the modern view of the structure and bonding of these compounds, as well as some of their distinctive chemical reactions.

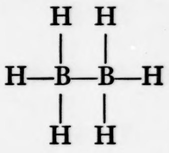
In the century that has elapsed since the idea of chemical structure—in the sense of definite linkages between atoms—began to be accepted, our understanding has increased to such a point that knowledge of the physical state of a substance and of the geometrical arrangement of the atoms in it enables us to give a fairly adequate description of the nature of the chemical bonds present in the substance. The partial dispersion of the veil of mystery concealing the nature of chemical bonds has been due to the efforts of many scientists. Among early workers the names of Butlerov [1], Kolbe, Frankland, and Kekulé come quickly to mind. Among the pioneers the name of G. N. Lewis [2] occupies a special position, for it was he who laid the foundations of the electronic theory of valence, recognizing that stable shells of electrons could be created by the transfer of electrons from one atom to another, forming ions, or by the sharing of two electrons between two atoms, forming a covalent bond. These ideas were extended and sharpened by others, notably I. Langmuir, N. V. Sidgwick, and L. Pauling.

For the majority of covalent substances the electron-pair theory of valence, refined by the application of quantum mechanics, and underlain by the assumption that each bond involves two atoms only, has reasonable validity. However, contemporary with the birth of the electronic theory of valence was the identification of a class of covalent substances that did not fit within the framework of the electron-pair theory of valence. These substances were a series of volatile boron hydrides [3].

Because boron is in Group III of the periodic classification of the elements, the formula of its simplest molecular hydride should be BH_3 (borane); instead, the simplest molecular hydride has the dimeric formula B_2H_6 (diborane). The properties of diborane, such as the boiling point of $-92{\cdot}5°$ C, are in accord with the presence in the hydride of essentially covalent bonds. However, a structure in which all the linkages are conventional electron-pair bonds requires at least fourteen valence electrons to hold the hydride together. In diborane the number of valence electrons is only twelve. The molecule is therefore said to be 'electron-deficient', this term being used to describe any compound having too few valence electrons to permit all atoms to be held together by two-atom electron-pair bonds.

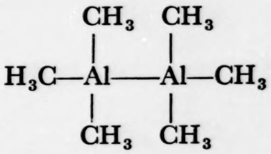
The property of electron-deficiency is not limited to the hydrides of boron. Discovery that trimethylaluminium is dimeric [4] and that tetramethylplatinum is tetrameric [5] led to the realization that a few substances having metal–carbon bonds were also electron-deficient. As with diborane, the polymeric character of trimethylaluminium and tetramethylplatinum implies the existence in these substances of an excess of bonds over electron-pairs.

For many years the study of electron-deficient compounds was hampered by the fact that chemists were led into attempts to explain geometrical structures that were, in fact, incorrect. Thus, the electron-diffraction technique, less reliable than at present, gave results which appeared to favour an ethane-like structure (I) for diborane, and a structure (II) for the dimer of trimethylaluminium like that of hexamethylethane:



$$\begin{array}{cccc} & H & H & \\ & | & | & \\ H- & B- & B & -H \\ & | & | & \\ & H & H & \end{array}$$

I



$$\begin{array}{cccc} & CH_3 & CH_3 & \\ & | & | & \\ H_3C- & Al- & Al & -CH_3 \\ & | & | & \\ & CH_3 & CH_3 & \end{array}$$

II

The correct configuration of the atoms in diborane (figure 1) was first established by a study [6] of its infra-red spectrum, and subsequently confirmed by a variety of other physical evidence [7]. The dimer of trimethylaluminium has a

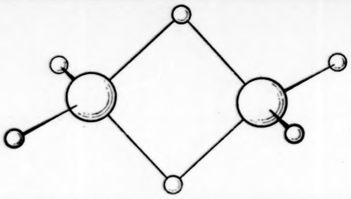


FIGURE 1 – *The atomic arrangement in diborane* (B_2H_6).

structure [8] analogous to that of diborane, with methyl groups instead of hydrogen atoms, and aluminium atoms instead of boron atoms. Establishment of the dispositions of the atoms in electron-deficient compounds is of paramount importance, since without knowledge of geometrical structures any discussion of the distribution of electrons between atoms is useless. It is fortunate, therefore, that during the last decade rapid progress in the determination of the structures of electron-deficient compounds has been made by the X-ray diffraction method, principally by W. N. Lipscomb [9] and R. E. Rundle [10] and their respective co-workers.

The feature common to all electron-deficient compounds is an atom, usually of a metal, having fewer valence electrons than stable bonding orbitals, and occurring in combination with atoms or groups containing no unshared electron-pairs. In these circumstances, classical valence theory, with its assumption that a covalent bond is a linkage localized between two atoms only, is quite unsatisfactory. In an electron-deficient compound, delocalization of the electrons occurs so as to employ all of the low-energy orbitals of the metallic atom in bonding. This situation leads to a relaxation of the stereochemical restrictions that govern the disposition of atoms in electronically saturated molecules. These geometrical restrictions are imposed because, in atoms with all valence orbitals filled, the different electron-pairs tend to avoid one another as far as is possible. Thus, as has been pointed out by Pauling [11], in most electron-deficient compounds the metal atom has a ligancy (co-ordination number) not only greater than the number of valence electrons, but also greater than the number of low-energy orbitals. Moreover, atoms adjacent to an electron-deficient atom increase their co-ordination number to a value above that of their orbital number.

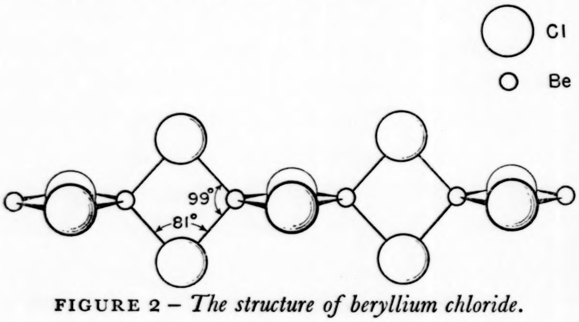


FIGURE 2 – *The structure of beryllium chloride.*

These principles may be illustrated by a consideration of the structures of beryllium chloride and dimethylberyllium, the latter, but not the former, being an electron-deficient compound. In many beryllium compounds, the beryllium atoms form four bonds that tend to be directed towards the corners of a tetrahedron, as in solid beryllium chloride, a chain polymer (figure 2) [10]. A beryllium atom in the gas phase in its lowest energy-state has four electrons, two being paired in the 1s orbital and two being paired in the next most stable orbital, the 2s, so that the electronic configuration of the atom is described as $1s^2 2s^2$. This description of the electron distribution in a free beryllium atom, involving a filled inner shell and a filled sub-shell, is clearly of no help in explaining the structure of beryllium chloride. Difficulties of this type have been circumvented by the application of quantum-mechanical principles and the concept of hybridization of bond orbitals [11]. For an electron associated with an atom, three 2p atomic orbitals correspond to the next energy level above the 2s orbital. It is found that a linear combination of these four orbitals yields bond orbitals of lower energy than those obtainable from s or p orbitals alone. The four so-called hybrid orbitals, the maximum number permissible in the valence shell of beryllium, are disposed tetrahedrally in space. However, a beryllium atom, possessing only two valence electrons, requires six more electrons from other atoms or ions to attain the especially stable arrangement of a completed electron-shell. In a monomeric beryllium chloride molecule, the valence shell of the beryllium atom, which must share its electrons with two chlorine atoms, contains only four electrons. In the gas phase, at elevated temperatures, it is possible for the monomeric species $BeCl_2$ to exist. In this event each beryllium–chlorine linkage can be regarded as an electron-pair bond, represented as being formed by overlap of a beryllium sp-hybrid orbital with a p-orbital of one of the chlorine atoms. Now a chlorine atom forming a single bond with another atom possesses non-bonding electron-pairs in p-orbitals. These electron pairs can be used to link other atoms, provided the latter have vacant orbitals available. The linkage formed in this

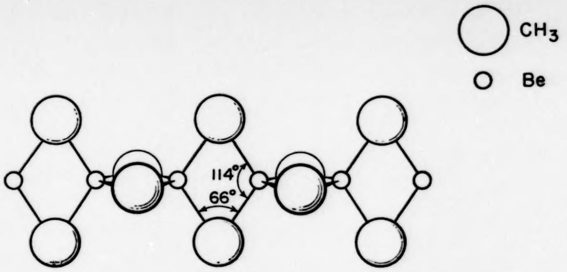
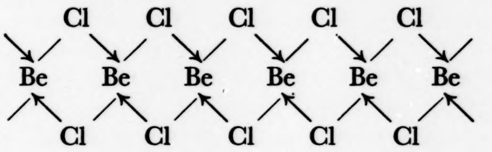


FIGURE 3 – *The structure of dimethylberyllium.*

manner is a dative bond, with both the electrons necessary for bond formation supplied by one atom. Since in monomeric beryllium chloride the beryllium atom does not have a completed shell, and since beryllium can have a total of four valence-orbitals, it is not surprising that, under normal temperature conditions, the monomeric species $BeCl_2$ polymerizes (III) so as to use all low-energy (valence) orbitals of beryllium, giving both beryllium and chlorine completed electron-shells. The resultant geometrical structure[1] is perforce a compromise between the four beryllium orbitals, which ideally would be tetrahedrally disposed, and the two chlorine orbitals, which ideally would be at right angles. Halogen bridges of the type found in beryllium chloride are fairly common in the metal halides. Thus the trichlorides of aluminium and indium are dimers in the gas phase, and the palladium chloride $PdCl_2$ is a polymeric solid. It is important to note that these polymeric halides are not electron-deficient, since every metal-halogen bond contains an electron-pair. Although the metals have more orbitals than valence electrons, electron-deficiency does not occur, because the halogen atoms bonded to the metals are able to contribute additional electrons.



III

The situation is very different in solid dimethylberyllium (figure 3), of which the vapour pressure is 0·6 mm at 100° C. In this compound, methyl groups serve as bridges between beryllium atoms [10]. Since methyl groups, unlike chlorine atoms, have no electron-pairs available for donation, the beryllium-carbon bonds cannot be treated as localized electron-pair bonds, and dimethylberyllium is electron-deficient. The co-ordination number of carbon has been raised to five, a value unusual for carbon, and, although the nearest neighbours of each beryllium atom are four methyl groups, the acute-angled group, Be—C—Be, has allowed adjacent beryllium atoms to approach closely enough to suggest some metal–metal interaction and also a ligancy of six for beryllium.

BONDING IN ELECTRON-DEFICIENT COMPOUNDS

The distribution of electrons in electron-deficient compounds has been the subject of much speculation for almost fifty years. Only during the last decade, however, has a reasonably satisfactory rationale of the bonding in these compounds been given. The modern viewpoint is based on a consideration of molecular orbitals rather than atomic orbitals.

A fundamental principle of the molecular-orbital theory of valence, as applied to a diatomic molecule AB, is that two molecular orbitals can be derived by a linear combination of an atomic orbital from atom A with an atomic orbital from atom B.[2] Of the two resultant molecular orbitals, one is of lower energy (bonding) and one of higher energy (antibonding) than either of the two original atomic orbitals (figure 4*a*). The electron-pair will occupy the orbital of lowest energy, the bonding orbital. Similarly, when three atomic orbitals on three centres are able to overlap, one obtains three molecular orbitals of which one

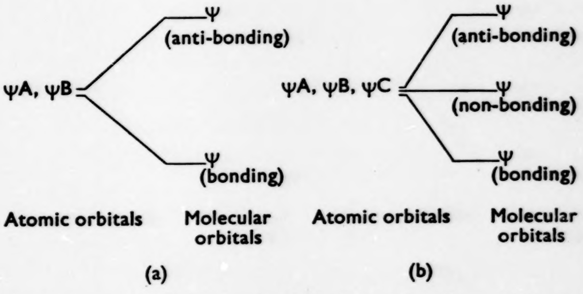


FIGURE 4 – *Molecular orbital formation from* (a) *two, and* (b) *three atomic orbitals.*

---

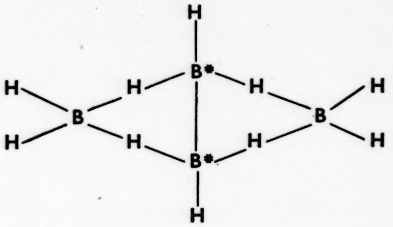
[1] Although the formula as given represents some bonds as donor bonds and others as ordinary covalent bonds, it should be recognized that it is not in fact possible to distinguish between the bonds.

[2] The approximation of the linear combination of atomic orbitals is applicable if the two atomic orbitals on the two atoms have approximately the same energies and the same symmetry relative to the molecular axis AB, and the charge clouds overlap sufficiently.

only is strongly bonding (figure 4*b*). Again there is only one state of lowest energy, and for this ground state only a single electron-pair is required, although three atoms are involved.

We are now in a position to see how the molecular-orbital (MO) correlation diagram for a three-centre bond [12] (figure 4*b*) can be applied to the electron-deficient B_2H_6 molecule [9, 13]. The 2s and three 2p atomic orbitals of the two boron atoms are hybridized to give each boron atom a set of four equivalent tetrahedrally disposed $2sp^3$-orbitals. Each boron atom uses two of its sp^3-hybrid orbitals to attach two hydrogen atoms. These are the four terminal hydrogen atoms, and the B—H bonds are of the normal electron-pair type, a fact suggested by force-constant measurements. Two sp^3-orbitals remain on each boron atom, and each bridging hydrogen atom possesses a 1s-orbital, so that six atomic orbitals have still to be considered. For the filling of these orbitals, four valence-electrons remain, eight having been used in the terminal B—H links. The B—H—B bridges may then be described in terms of two localized molecular orbitals, each formed by combination of two sp^3 hybrid atomic orbitals on the two boron atoms and a 1s-orbital of hydrogen. Since a linear combination of atomic orbitals from three atoms leads to a unique lowest-energy state (figure 4*b*), the four valence electrons, divided into two pairs, are sufficient to fill the two 'three-centre' bonds that are concentrated on either side of the boron-boron axis (figure 5*a*). The polyatomic diborane molecule can thus be described in terms of filled valence-orbitals by invoking the concept of two localized molecular-orbitals extending over three atomic nuclei. Clearly, such three-centre bonds involve more electron delocalization than the normal two-centre bond.

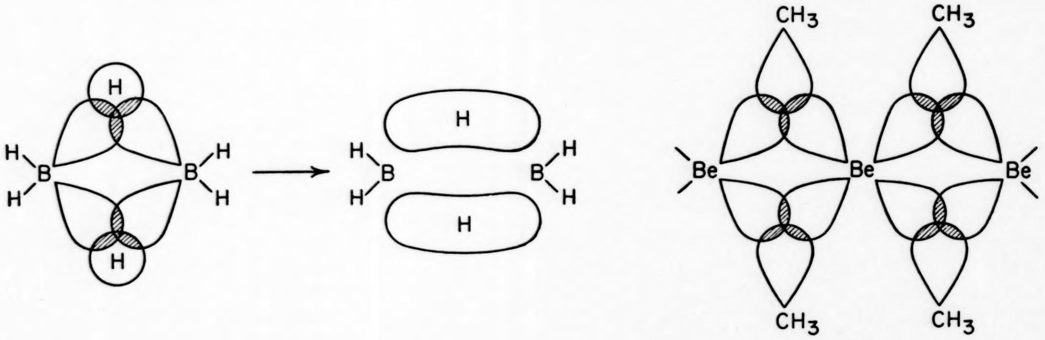
Three-centre bonds can also be used to describe the bonding in some of the higher boron hydrides. Consider, for example, tetraborane (figure 6), again assuming sp^3-hybridization for boron. The hydride B_4H_{10} has twenty-two valence electrons.



IV

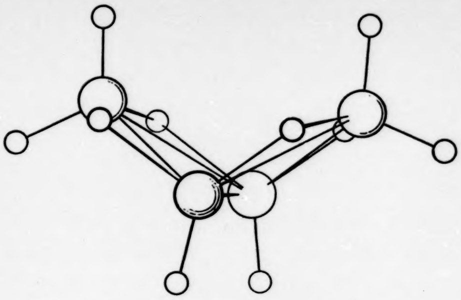
Six hydrogen atoms are bonded to boron atoms by six electron pairs, and the four B—H—B three-centre bonds (IV) require four electron pairs. These ten pairs of electrons fill all the hydrogen orbitals and all the boron orbitals, except for one orbital on each of the two boron atoms B* (IV). There remains one electron pair for these two boron orbitals that are directed towards one another and can therefore form an electron-pair B—B bond. The same bonding principle can be used for the hydride B_5H_{11}, except that it is necessary to consider two three-centre bonds of the type B—B—B as well as three bonds of the type B—H—B. It is interesting to note the diborane type of geometry that occurs in the open parts of the higher boron hydrides (figure 6).

Molecular orbitals localized in the sense that three atom-centres are considered separately from other atoms in the molecule are also a useful concept for describing the bridge bonding in the trimethylaluminium dimer and polymeric dimethylberyllium (figure 5*b*). A single stable localized molecular orbital is obtained by combining an sp^3-hybrid carbon orbital with sp^3-orbitals of adjacent aluminium or beryllium atoms. An

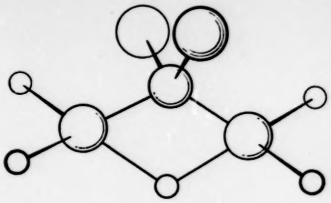


(a) (b)

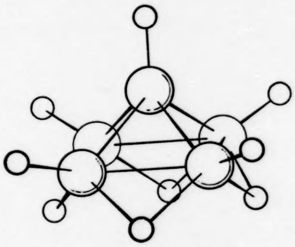
FIGURE 5 – (a) *Bridge bonding in diborane.* (b) *Bridge bonding in polymeric dimethylberyllium.*



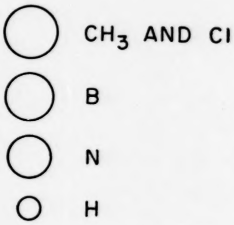
The structure of B_4H_{10}.

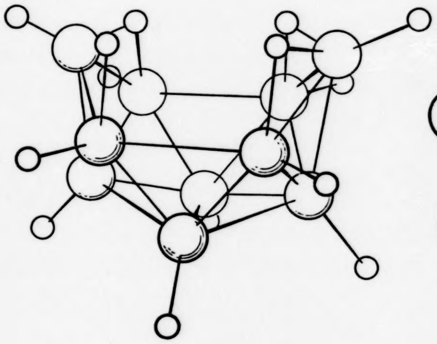


The structure of $(CH_3)_2NB_2H_5$.

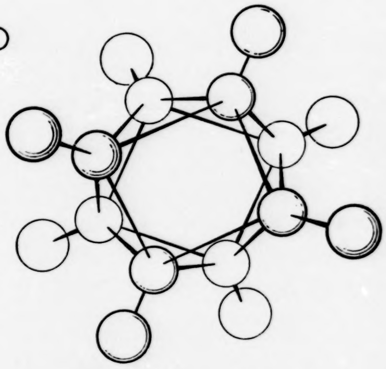


The structure of B_5H_9.





The structure of $B_{10}H_{14}$.



The structure of B_8Cl_8.

FIGURE 6 – *Electron-deficient boron compounds.*

electron-pair is then available for the filling of the three-centre bonds.

In electron-deficient compounds that are more complex than those mentioned above, greater electron delocalizations occur, and the three-centre-bond concept has to be abandoned in favour of multicentre bonds. Thus in tetramethylplatinum (figure 7) a four-centre orbital is required to bond a methyl group to three platinum atoms using one electron pair. Similarly, in the pentaborane B_5H_9 (figure 6) it is necessary to assume a five-centre orbital, involving the five boron atoms in addition to three-centre and other orbitals, in order to obtain a satisfactory description of the molecule.

R. E. Rundle has pointed out [10] that the electron delocalization that is characteristic of electron deficiency increases in a continuous manner as the ratio of valence orbitals to valence electron-pairs increases. One example of this tendency is afforded by the hydrides of boron and aluminium. Like its Group III congener boron, aluminium possesses three valence electrons. However, elements in the row below boron in the Periodic Table are not limited to four valence orbitals but may form up to six. Thus it is not surprising that whereas the BH_3 group dimerizes to B_2H_6, the AlH_3 group displays greater electron-deficiency, forming a highly polymeric solid $(AlH_3)_n$. Similarly, lithium alkyls are more polymeric and therefore more electron-deficient than trimethylaluminium. The greater electron-deficiency of covalent lithium compounds is to be expected, because in a covalent compound, with the implied principle of electron sharing, lithium has but one valence electron to contribute to a total of four possible orbitals.

Metals themselves can be regarded as highly electron-deficient. There are so many orbitals and so few electrons that the electron-pair theory of valence with its localized bonds has little meaning, and other theories have to be advanced to account for metallic bonds.

FURTHER EXAMPLES OF ELECTRON-DEFICIENT COMPOUNDS

To emphasize the widespread occurrence of electron-deficiency, some further examples may be cited.

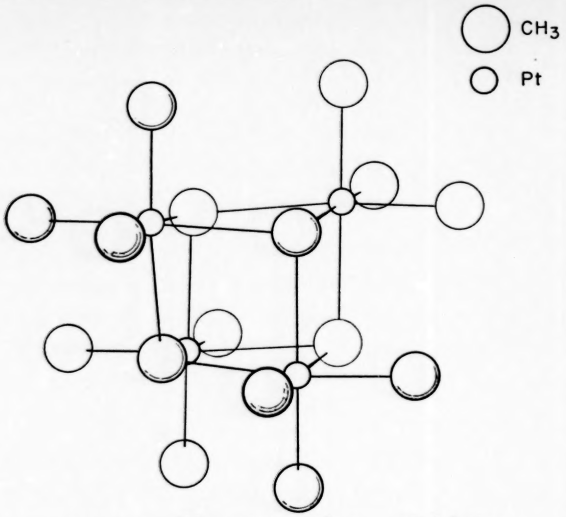


FIGURE 7 – *The structure of tetramethylplatinum.*

Nearly all the known boron hydrides form derivatives in which one or more hydrogen atoms are replaced by other atoms or groups [14]. Bromodiborane (B_2H_5Br), for example, has a structure analogous to diborane, with one of the terminal hydrogen atoms replaced by a bromine atom. Numerous substitution derivatives of decaborane (figure 6) are also known, such as $B_{10}H_{12}(NCCH_3)_2$ and $B_{10}H_{13}I$ (two isomers reported). Substituted boron hydrides of this type contain B—H—B bridges and are electron-deficient, just as are ions derived from the hydrides (such as $B_{10}H_{13}^-$ and $B_3H_8^-$), and certain compounds (such as $(CH_3)_3N.B_3H_7$) obtained by treating boron hydrides with electron-pair donors.

There are many other substances having metal–hydrogen–metal bridges, among them the covalent members of the class of compounds called borohydrides that has been studied over the past 20 years by H. I. Schlesinger and his co-workers. The covalent borohydrides, such as $Be(BH_4)_2$, $Al(BH_4)_3$, $Ti(BH_4)_3$, and $U(BH_4)_4$, have boron–hydrogen–metal linkages, and, for substances containing metals, are exceedingly volatile. Aluminium borohydride, for example, boils at 44·5°C.

Related to the covalent borohydrides, in the sense that they have hydrogen-bridge bonds, are certain polymeric hydrides. Aluminium hydride, mentioned earlier, is representative of this class of hydride, and other examples include hydrides of beryllium, zinc, and cadmium. These polymeric hydrides, with their large degree of cross-linking of metals through hydrogen, are highly electron-deficient. On the other hand, substances that are electron-deficient only to a limited extent are exemplified by the thiodiboranes (RSB_2H_5) and the aminodiboranes ($R_2NB_2H_5$) described by A. B. Burg and his co-workers [16]. In these compounds, such as dimethylaminodiborane [$(CH_3)_2NB_2H_5$] figure 6, only one electron-pair three-centre B—H—B bond is present. The B—S—B and B—N—B bridges are not electron-deficient, since electron-pairs on sulphur and nitrogen can donate to adjacent boron atoms, just as lone pairs on chlorine donate to beryllium in polymeric beryllium chloride (III). In other cases, the occurrence of electron-deficiency depends on the physical state. Thus trimethylindium (m.p. 88·4°) is monomeric as vapour but polymeric as a solid [17].

An interesting example of electron-deficiency is provided by the boron halides B_4Cl_4 and B_8Cl_8 [9], which are highly condensed molecules so that, as mentioned earlier, use of three-centre orbitals is not satisfactory, and a description based on multicentre molecular orbitals is to be preferred. The boron atoms in B_4Cl_4 are linked together tetrahedrally, and each is bonded to a chlorine atom lying outside the tetrahedron. The structure of B_8Cl_8 involves an interesting polyhedron of boron atoms (figure 6). In B_4Cl_4 the B—Cl bonds may as a first approximation be regarded as electron-pair bonds, leaving eight valence electrons available to hold the four boron atoms together. It is possible to show [13] that the atomic orbitals remaining on the tetrahedrally arranged boron atoms can be compounded into six bonding molecular orbitals. The first four of these orbitals employ the eight available valence electrons, while the remaining two have the correct symmetry to accept electrons from the filled p-orbitals possessed by the chlorine atoms. A filled-orbital description of the halide is thus possible. A somewhat similar description can be given to the bonding in B_8Cl_8 [9] and to the bonding in certain electron-deficient borides that contain octahedra of boron atoms, such as CaB_6 [13].

CHEMICAL REACTIVITY

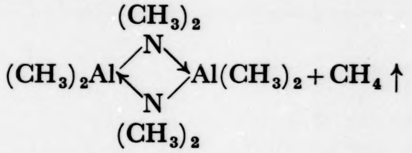
In general, electron-deficient compounds react readily with substances able to donate electrons, the 'nucleophilic reagents' or 'Lewis bases'. Such reactions can give a wide variety of products, and many hundreds of interesting compounds have been obtained from electron-deficient substances in this manner. This short article can, of necessity, list only a few reactions of this kind.

Trimethylaluminium and dimethylberyllium are depolymerized by amines and phosphines, forming addition compounds

$$(CH_3)_3N \rightarrow Be(CH_3)_2 \quad \text{or} \quad (CH_3)_3P \rightarrow Al(CH_3)_3$$

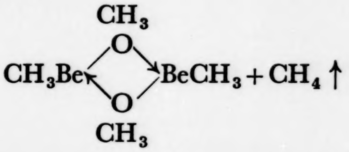
in which all bonds are of the electron-pair type. If the donor atom carries a hydrogen atom, the initial reaction product releases methane on heating:

$$(CH_3)_2NH.Al(CH_3)_3 \xrightarrow{\text{heat}}$$



Sometimes treatment of an electron-deficient compound with a Lewis base leads to so rapid and vigorous a reaction that no intermediate co-ordination compound is detected:

$$[(CH_3)_2Be]_n + CH_3OH \longrightarrow$$



The electron-deficient metal alkyls react with water with explosive violence, probably through intermediates such as $H_2O \rightarrow Al(CH_3)_3$.

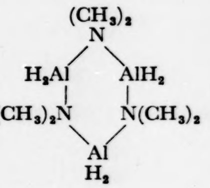
The chemistry of the boron hydrides has received extensive study [14], and most reactions may be assigned to two main classes. Either a borane group may be removed from a boron hydride structure, as in

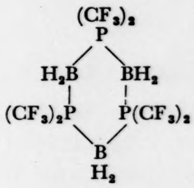
$$B_2H_6 + 2CO \xrightarrow{\text{pressure}} 2OC.BH_3$$

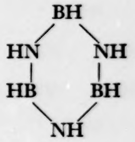
or a BH_2^+ group is removed, as in

$$B_4H_{10} + 2NH_3 \xrightarrow{\text{low temperature}} [H_2B(NH_3)_2]^+(B_3H_8)^-$$

Reactions of boron hydrides involving removal of BH_3 groups are so common that there are some 50 known addition compounds of the type $L.BH_3$, where L is an electron-pair donor. A few analogous complexes of the AlH_3 group are also known, such as $(CH_3)_3N \rightarrow AlH_3$. Unlike the borane compounds, the AlH_3 co-ordination compounds are associated to some extent in organic solvents. Association probably involves Al—H—Al bridges, with aluminium using more than four orbitals (see above), so that the compounds are still electron-deficient to some extent. Frequently an electron-donor molecule will form an AlH_3 or BH_3 addition compound that will subsequently decompose, under appropriate conditions, with release of hydrogen. The following are representative of a large number of compounds made in this way.







REFERENCES

[1] Butlerov, A. M. *Z. Chem. Pharm.*, **4**, 549, 1861.

[2] Lewis, G. N. *J. Amer. chem. Soc.*, **38**, 762, 1916.

[3] Stock, A. 'Hydrides of Boron and Silicon'. Cornell University Press, Ithaca, New York. 1933.

[4] Laubengayer, A. W. and Gilliam, W. F. *J. Amer. chem. Soc.*, **63**, 477, 1941.

[5] Rundle, R. E. and Sturdivant, J. H. *Ibid.*, **69**, 1561, 1947.

[6] Price, W. C. *J. chem. Phys.*, **16**, 894, 1948.

[7] Stone, F. G. A. *Quart. Rev. chem. Soc., Lond.*, **9**, 174, 1955.

[8] Lewis, P. H. and Rundle, R. E. *J. chem. Phys.*, **21**, 986, 1953.

[9] Lipscomb, W. N. *Advanc. inorg. Chem. Radiochem.*, **1**, 146, 1959.

[10] Rundle, R. E. *J. phys. Chem.*, **61**, 45, 1957.

[11] Pauling, L. 'The Nature of the Chemical Bond' (Third edition). P. 363. Cornell University Press, Ithaca, New York. 1960.

[12] Longuet-Higgins, H. C. *J. chim. phys.*, **46**, 275, 1949.

[13] *Idem. Quart. Rev. chem. Soc., Lond.*, **11**, 121, 1957.

[14] Stone, F. G. A. *Advanc. inorg. Chem. Radiochem.*, **2**, 279, 1960.

[15] Schlesinger, H. I., Brown, H. C. *et al. J. Amer. chem. Soc.*, **75**, 186, 1953.

[16] Burg, A. B. *Rec. Chem. Progr.*, **15**, 159, 1954.

[17] Amma, E. L. and Rundle, R. E. *J. Amer. chem. Soc.*, **80**, 4141, 1958.

Space research

SIR HARRIE MASSEY

Because of both its very high cost in terms of money and skilled manpower, and the uncertainty about its potential value, space research has inevitably excited a good deal of controversy. This article considers what is meant by space research, what it entails, and what we may reasonably hope to learn from it. Particular attention is paid to distinguishing the roles of the two principal space vehicles, vertical sounding rockets and satellites.

THE MEANING OF SPACE RESEARCH

The availability of rockets with high-power motors, and of accurate methods of guiding, controlling, and tracking them, has far-reaching consequences, but there is at present much confusion about the meaning of the term 'space research'. To many, the first meaning that springs to mind is space travel—voyaging by man in space, including landings on the Moon and planets. Such journeys have so romantic an appeal, representing such an outstanding challenge to man's ingenuity, endurance, and adaptability, that many consider no other justification to be necessary. Other proponents of manned space-travel suggest great advantages to be gained from it, ranging from the colonizing of celestial objects, thereby relieving the problem of over-population, to the possibility of tapping new sources of mineral and other wealth. Still others would say that through manned travel we can add greatly to our knowledge of the universe around us and particularly about our nearest neighbours, the Moon, Venus, and Mars. They point out that one of the most fascinating problems of all—to what extent life is confined to this planet—might well find an answer if Mars or Venus could be explored. Such knowledge would not only be of the greatest interest for its own sake but might have applications whose consequences are quite unforeseeable. We must remember, however, that much research may be carried out automatically by instruments carried in a space vehicle, making measurements and transmitting the results back to Earth by means of coded radio signals. So much can be done in this way that it is at least questionable whether the great additional effort and expense necessary to put man safely into space would be justified for scientific observation alone. This does not in itself imply, of course, that the present effort being made to realize manned space-travel is unjustified, for this involves other considerations than scientific research.

It is the scientific research made possible by the availability of space vehicles that the writer prefers to call space research, but he is well aware that this is a purely personal choice and that the term is often employed in a much wider sense. For this reason there has been a tendency to use the new term 'space science' to apply only to the scientific research, but this also is gradually being extended in meaning to cover the whole field. In this article we shall stick to the narrower meaning and discuss no further the case for man in space. On the other hand, the new scope for scientific research will be considered in some detail, with reference also to commercial applications.

In advocating such space research we must be clear that the new knowledge to be gained by the use of automatic equipment carried in space vehicles is of wide range and importance. It is of great value, for example, to the astronomy of the Sun and stars; the physics of the Earth and planets; the study of solar-terrestrial relations; meteorology; the study of cosmic rays; and biophysics. Already there are possible applications of commercial importance, especially to world-wide radio communications and navigation, and as the work develops, there will doubtless be many more.

Before proceeding, one other point of confusion needs to be cleared up. This is one that occurs within the purely scientific part of the subject, and arises through failure to appreciate that space research, as we understand it, is a unity of technique, not of scientific discipline. It is not considered, for example, that radioastronomy, or indeed optical astronomy generally, is part of space research; only those parts of the subject that depend on observations made with equipment in space vehicles are so considered. The realization of this situation is important, as it would be most undesirable for the programme of space research to develop outside the scientific disciplines concerned. They must be closely integrated, and at all times care must be taken that elaborate and expensive space-vehicles are not used to obtain results that could be derived adequately by the use either of entirely ground-based techniques or of balloons. Equally, it is necessary for scientists not directly concerned in space research to be thoroughly aware both of the contributions already made to their subject by it and of the possibilities it affords for further investigations. To realize the potentialities fully it is necessary to set up laboratory programmes directed on the one hand towards the development of appropriate new techniques of measurement, and on the other to supplementing and interpreting information from space research.

TYPES OF SPACE-RESEARCH VEHICLE

We have already implicitly and arbitrarily indicated that we do not regard a balloon as a space vehicle. Conforming to the idea of a unity of technique, we regard as a space-research vehicle one that requires, at some stage or other, rocket propulsion independent of the existence of a surrounding atmosphere. Within this category it is best to distinguish between vehicles that pursue nearly vertical trajectories, returning to earth within a few minutes of launching, and those that leave the Earth's surface for long periods, and even for ever, as artificial satellites or deep-space probes.

All these classes of vehicle have an important role to play. The vertical sounding rocket has the advantage of relative cheapness, but suffers from the shortness of the period during which observations may be made. Many of the properties and phenomena of the upper atmosphere vary greatly according to place and time, and it therefore requires an enormous number of vertical probing flights to obtain a thorough knowledge of any of them. Further, there are weak effects that cannot be detected in a short flight and can be studied only by long-term observation. The use of artificial satellites, when possible, obviates these difficulties. Thus a satellite circulating for a month can provide as much data as eight thousand vertical sounding rockets using similar equipment: there is nearly world-wide coverage, and both sporadic and weak effects can be studied effectively. On the other hand, a satellite cannot circulate for long at altitudes much below 150 km because of air drag; this quite soon brings the orbit within the dense lower atmosphere, where the satellite burns up.

It follows that vertical sounding rockets are essential for studies of the atmosphere between the upper limit attainable by balloons (about 30 km) and 150 km. To overcome the chief disadvantage of such rockets, attention must be devoted to simplifying their design and launching, so that they may be fired frequently from many locations, including ones over the sea. Full advantage can be taken of such developments only if there is a corresponding reduction in the size and weight of the scientific equipment necessary to make any particular kind of observation.

For studies at altitudes above 150 km and requiring systematic observations over considerable periods—and this applies to most investigations—artificial satellites are the appropriate vehicles. There are some phenomena, however, that may be adequately studied at altitudes above 150 km even in the short time of a rocket flight; certain cosmic-ray and radioastronomical measurements are of this kind. In such cases it is not economical to use satellites.

For most purposes it is not necessary to recover either the instruments or records of any kind, the data obtained in flight being transmitted back to receiving stations on the ground in the form of coded radio signals. But recovery is desirable in those cases in which it is an advantage to use photographic film. There is no serious problem involved in doing this after a vertical sounding flight, but it is much more difficult to bring back equipment within a satellite. However, as this is a problem that must be solved before man can venture forth in a satellite, the Soviet Union and the United States have studied it closely, and both have achieved successful recoveries.

To explore the region between the Earth and the Moon (cislunar space) and to study the Moon and planets, it is necessary to launch vehicles at speeds close to the escape value (25 000 miles per hour). Such vehicles may be arranged to pass round the Moon and return to the near neighbourhood of the Earth; to hit the Moon; to pass close to the Moon and then continue on to become an artificial planet; to become an artificial lunar satellite; or merely to pursue a highly eccentric elliptic orbit round the Earth so as to penetrate at the apogee to distances of several earth radii into cislunar space. The possibilities may be enlarged considerably if one includes 'soft' lunar landings and planetary journeys similar generally to those we have listed for the Moon. One specially interesting and important case is that of a satellite stationary with respect to points on the Earth's surface. This will be the case for a satellite revolving in a nearly circular orbit in the equatorial plane with a period exactly equal to that of the Earth's rotation on its axis, a condition satisfied if the satellite is at a distance of about 23 000 miles from the Earth's surface.

TECHNICAL REQUIREMENTS

Apart from the obvious need for sufficiently powerful rocket motors and accurate systems of guidance and control, there are many other technical requirements. Some of these are of a general character common to all space research, while others are peculiar to the actual experiments that determine the payload of the vehicle concerned.

We have already mentioned the necessity of providing means for accurate tracking after launching. The most precise means of doing this is to use optical methods when these are applicable, but in many circumstances it is necessary to rely on radio direction-finding based on a transmitter within the vehicle. An allied problem is that of telemetry, that is, the transmission of data by means of coded radio signals from the vehicle. It is clearly necessary to extend as far as possible the range both of useful radio direction-finding and of data transmission. To assist in this, the necessary equipment should be both sensitive and capable of high discrimination between the wanted signal and background noise. The recent trial carried out jointly by the National Aeronautics and Space Agency of the United States and the Jodrell Bank Experimental Station has shown what can now be achieved. Not only were signals received from a space vehicle up to a distance of 22 000 000 miles, but the transmitter in the vehicle was switched on by command signal from Jodrell Bank when at a distance of 8 000 000 miles.

Another vital technique that must be developed is that of controlling the temperature of the vehicle so that it remains within the range in which the instruments on board will operate adequately. This problem is not very serious with vertical sounding rockets, but is important for satellites. The temperature at any time depends essentially on the balance between emission and absorption of radiation. Not only must it be controlled within prescribed limits but it must not fluctuate too much as the satellite moves in and out of sunlight. Much progress has been made in dealing with this problem by coating satellites with material of appropriate emissive properties. The problem is more severe for a vehicle penetrating to the neighbourhood of Venus, as the intensity of sunlight appreciably increases in this closer approach to the Sun. As indicated earlier, recovery of a satellite is difficult, and one of the main difficulties is the severe heating resulting from atmospheric resistance; here the temperature-control problem is much more serious.

Power must be supplied within a space vehicle to operate the measuring instruments and the radio transmitters. Here again there is no difficulty in a vertical sounding rocket, as conventional batteries may be used; but for satellites designed to circulate for a year the necessary battery capacity can be obtained only at the cost of a prohibitive increase in total payload. One effective solution is to use cells activated by sunlight. Another suggestion is to make use of radioactive sources, and there is clearly a great incentive to extensive research in this direction.

Little space research could be done if it were not for the availability of high-speed methods of computation. These make it possible rapidly to predict from the early observations the future track of a fast-moving vehicle, and this is essential for guidance and control systems. An equally important application is in the analysis of the vast amount of data transmitted from equipment in a satellite that has been circulating for months. The importance of this cannot be exaggerated, for unless the desired information can be extracted rapidly from the original records the whole operation is made valueless.

Many other technical problems arise in considering in detail the contents of a space vehicle that is to perform selected observations. There is, of course, the requirement of high reliability of the equipment, without maintenance, and there is the need for keeping to a minimum the volume and weight of the different components. Countless other problems have also to be resolved. Adequate methods of laboratory testing of prototypes under simulated conditions of acceleration, temperature, and so on must be developed. At all stages there is great scope and need for technical ingenuity, and the solution of the widely varying, unusual, and difficult problems associated with space research is bound to lead to new techniques of value in many other directions. To demonstrate this one need only point to the question of reliability of automatic operation over long periods.

Proceeding now to discuss the nature of the possible applications to different aspects of pure scientific research, it is perhaps best to begin with the prospects space research affords for advancing the study of the Earth's atmosphere and then to move gradually outwards. This approach is not intended to suggest an order of importance.

THE EARTH'S UPPER ATMOSPHERE

Above the limit of about 30 km attainable by balloons the atmosphere extends for hundreds of kilometres before merging with interplanetary space. It is best to judge the extent to which the atmosphere is still present at any altitude not by its pressure but by the number of atoms, ions, or electrons present per unit volume. Although at 100 km altitude the air pressure is about one-millionth of that at ground level, there are still about 5×10^{13} atoms and molecules per cubic centimetre. At 300 km, where the air pressure has fallen to 10^{-11} times the ground value, there are still more than a million times as many atoms and molecules than there are neutral or ionized particles in the same volume of cislunar space.

The atmosphere at great heights is the site of many phenomena, some of which are of considerable importance for us on the ground. The quickest way of summarizing these phenomena is by relating them to the solar source—the Sun exerts a dominant influence on the atmosphere through the radiations it emits. The Moon, too, influences some phenomena by producing lunar atmospheric tides. As the Sun is a variable star, the nature and intensity of its radiation are not steady, but we may distinguish a general background, characteristic of the Sun when quiet, from the additional radiations emitted under disturbed conditions. Such disturbances are usually associated with variations in number and size of sunspots and are most frequent at intervals of eleven years. In fact, the solar activity, measured in terms of numbers of sunspots, goes through an eleven-year cycle.

The radiation characteristic of the quiet sun is electromagnetic, and its intensity in the visible region is what would be expected if the surface temperature were about 6000° C. Effectively, only the visible radiation reaches the ground; other wavelengths, both shorter and longer (except for narrow bands in the infra-red and radio waves between 1 cm and 15 m wavelength (see figure 1)), are absorbed at high altitudes and in consequence produce important atmospheric effects. These effects include the formation and maintenance of the ionosphere, which extends upwards from an altitude of 100 km to several times that height. The ionosphere is a region in which there is a considerable concentration of free electrons (10^5–10^6 per cubic centimetre), sufficient to affect markedly the propagation of radio waves longer than about 15 m, so that instead of passing out along straight paths into space they are reflected back to the ground, thus rendering possible long-distance radio communication. The supply of free electrons is maintained through ionization of atmospheric atoms and molecules by solar ultra-violet light and X-rays.

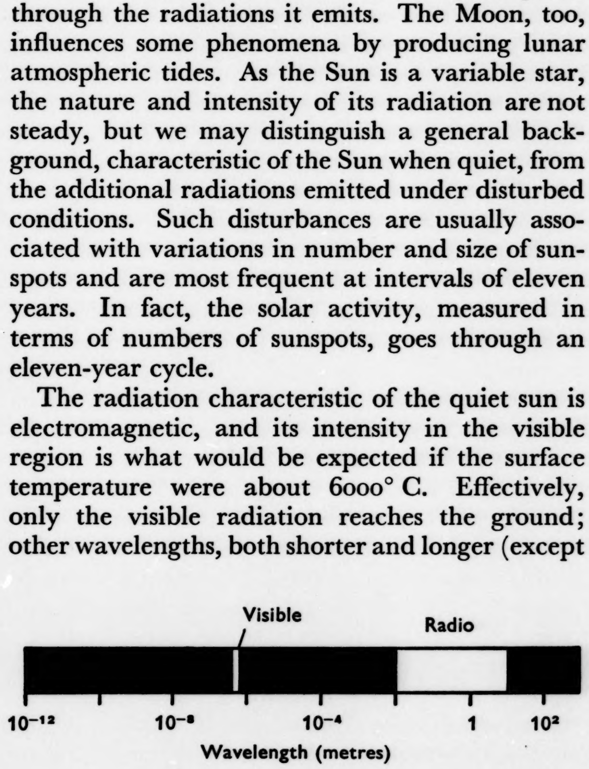


FIGURE 1 – *Spectrum of electromagnetic waves, showing the wavelengths (unshaded) that can penetrate the entire thickness of the Earth's atmosphere.*

Another remarkable effect of the steady ultra-violet radiation from the Sun is on the composition of the atmosphere. In consequence of photo-dissociation by these rays, oxygen exists predominantly in the atomic form at altitudes above 100 km. At lower altitudes, not far from the balloon limit, solar ultra-violet light produces photochemical effects that lead to a relatively high concentration of ozone. The ozone layer absorbs the remaining ultra-violet rays, which would otherwise be lethal for plant life on the ground.

These are just two of the major effects of solar radiation. There are many others, including the generation of the faint nocturnal airglow[1] and the quiet magnetic variations that arise from solar and lunar atmospheric tidal motions in the ionosphere.

The most conspicuous feature of the disturbed Sun is the emission of streams of charged particles, mainly protons and electrons, with speeds of about 1500 km/sec but at times including more energetic particles. Because of their charge, these particles are influenced by the Earth's magnetic field, so that they tend to enter the atmosphere in narrow belts located about 23° from either geomagnetic pole. In these regions they are responsible for the magnificent auroral displays, as well as strong disturbances of the Earth's magnetic field and of the ionosphere that may extend over a much wider latitude range. Most of the solar particles are not energetic enough to penetrate to within 80–100 km of the ground.

---

[1] The atmosphere at night emits a faint glow, known as the airglow, that is independent of auroral disturbances and is associated with photochemical reactions that arise from the effect of the Sun during the day.

Although we have merely indicated a few of the major aspects of the high atmosphere and of the nature of the solar influence, it should be apparent that there is a great deal of interest and importance to study. It is true that much can be done with entirely ground-based equipment, but if we depended on this alone many of the vital links in the chain of atmospheric processes would always remain obscure. Foremost among these is the solar radiation, which produces the atmospheric effects and, as it is absorbed in so doing, never penetrates into the lower atmosphere at all and cannot therefore be studied at ground level. We must have information about its nature and intensity before we can begin to understand the mechanism of production of the ionosphere, the airglow, the aurora, magnetic storms, and so on.

There are many other properties and phenomena that cannot be observed directly except with instruments taken to high altitudes. We need only cite the distribution of the positively charged particles in the ionosphere; the proportion of oxygen in atomic form at different altitudes; and the extent to which the atmospheric components separate out at different altitudes because of diffusion, to realize what a wealth of new possibilities arise, particularly when it is remembered that atmospheric properties vary with position over the surface, and with time of day, season, and position in the sunspot cycle. Furthermore, although information about atmospheric pressure, density, temperature, wind distribution, and electron concentration as a function of height can be derived—at least over certain altitude ranges—from observations on the ground, much more detailed and extensive information on all these variables can be obtained by direct measurement from a space vehicle.

The motion of a stream of charged particles from the Sun under the influence of the Earth's magnetism is so complicated as to defy theoretical prediction; but it is now possible, through the use of particle detectors and magnetometers carried in deep-space probes, to extend the study of the behaviour of these streams to great distances from the Earth. Perhaps the most important discovery yet made in space research has been that of the radiation belts surrounding the Earth (see figure 2). These are regions in which there is an unexpectedly high density of moving charged particles having energies of the order of a few tens of thousands of electron-volts. In some way the production and presence of these belts are associated with auroral and magnetic-storm phenomena. Although there is no fully adequate understanding of how the supply of particles is maintained, nor of how the particle concentration varies with solar activity, it seems fairly certain that the effect of the Earth's magnetic field in trapping particles moving with certain velocities is of major importance. The thorough study of the radiation belts is likely greatly to improve our knowledge of the way in which the Sun affects atmospheric phenomena. It can be carried out only through space research, and will take many years of systematic observations.

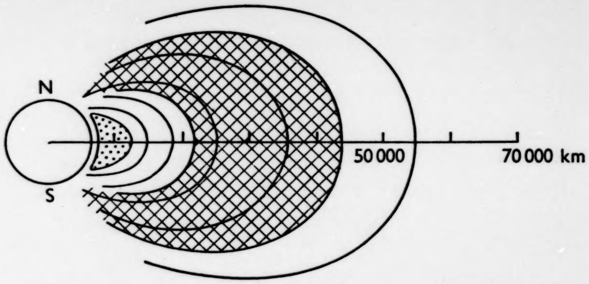


FIGURE 2 – *Natural radiation belts surrounding the Earth. The inner belt is indicated by stippling and the outer by cross-hatching; lines of force of the Earth's magnetic field are also shown. The outer belt is variable, and the region shown is based on observations made with equipment in Luniks I and II.*

PROPERTIES OF THE SOLID EARTH

The orbit of an artificial satellite is sensitive to the shape and constitution of the solid Earth, because this determines in detail the gravitational field acting on a body at any point. Already new data relevant to this have been obtained from analyses of the orbits of artificial satellites and much more will be learned as tracking methods become more precise and continuous. In this connection it will be possible to improve the accuracy of geodetic surveys—a matter of practical as well as scientific importance.

APPLICATIONS IN GENERAL ASTRONOMY

It has been pointed out above that only visible sunlight and certain solar radio-waves are able to penetrate the atmosphere, and this is necessarily true also of the radiation from the stars. Virtually all astronomical studies depend on observation of electromagnetic waves from heavenly objects, covering less than two octaves in the visible and about the same range in the radio region (figure 1). Observations made from outside the atmosphere are not so limited. They may be extended by many octaves to higher frequencies, through

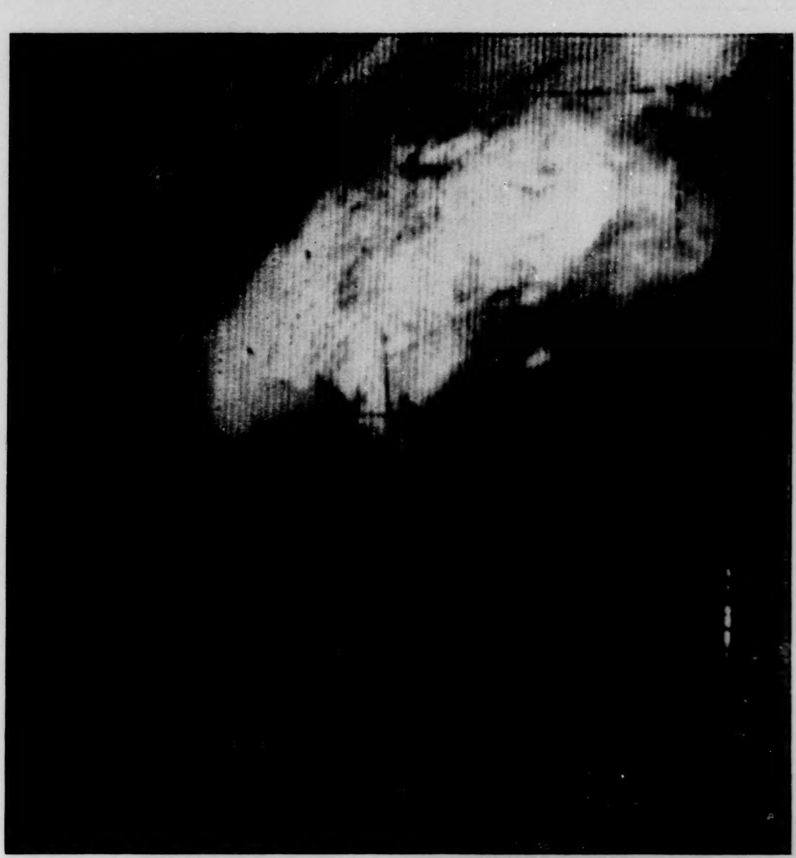
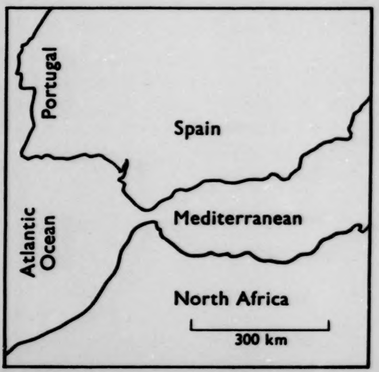
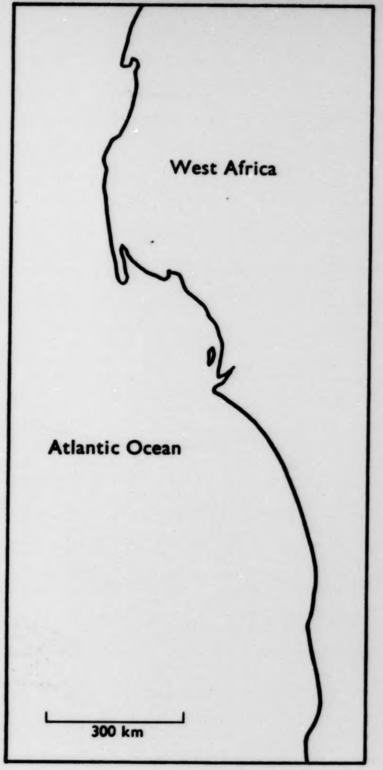


FIGURE 3 – *Photograph taken from Tiros I (launched 1st April 1960) while over North Africa, camera pointing downwards and to the west. The Straits of Gibraltar can be seen in the centre of the picture, with the Mediterranean to the right and the Atlantic to the left. Note cloud over Portugal.*


Portugal
Spain
Atlantic Ocean
Mediterranean
North Africa
300 km


West Africa
Atlantic Ocean
300 km

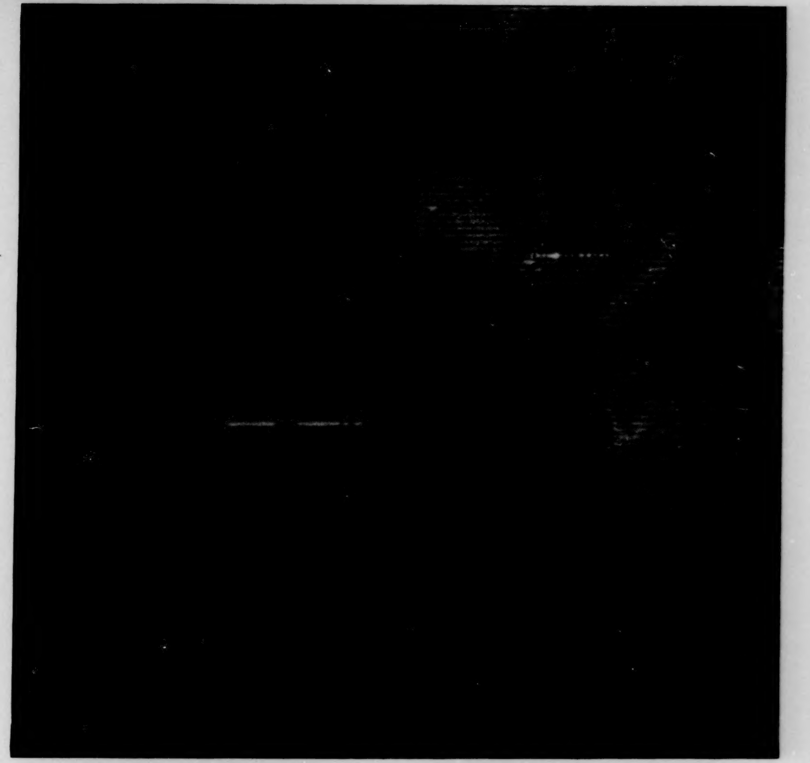


FIGURE 4 – *Part of the west African coast, approximately from Cape Blanco to St. Louis, photographed from Tiros I.*

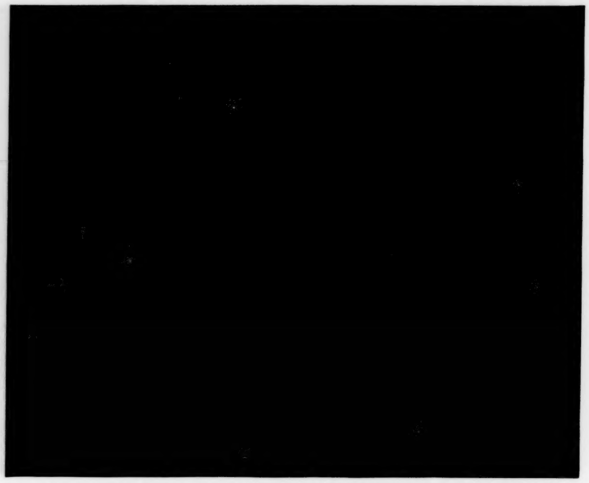


FIGURE 5 – *Photograph of the fluorescing trail of sodium vapour ejected from a Skylark rocket launched at twilight from Woomera, Australia. By studying the variation in the form of the trail with time, information may be obtained about the wind distribution at the height of the trail (between* 93 *and* 120 *km).*

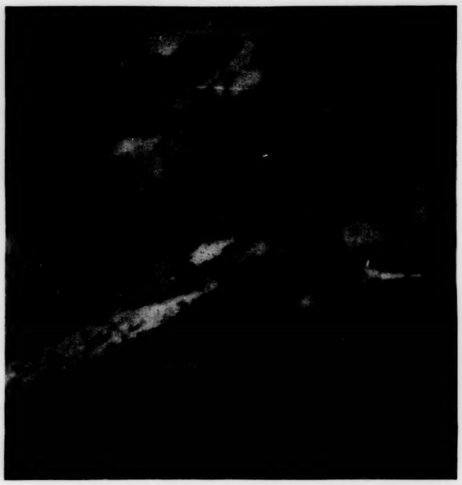


FIGURE 6 – *Photograph from Tiros I taken over the Red Sea, showing the Gulf of Suez and the Gulf of Aqaba, and eastern Mediterranean (top left). Cloud formations can be clearly seen across centre of picture.*

the ultra-violet and X-ray region to γ-rays, and equally to lower frequencies through the infra-red to long radio-waves (see figure 7). It is not possible to predict what will be discovered by means of telescopes or equivalent equipment, mounted in satellites, that view the universe in these hitherto inaccessible wavelength regions. Already preliminary scans of the northern sky have been carried out in ultra-violet radiation of wavelength 1500 Å (the shortest wavelength that can reach the ground is 2900 Å). Instruments in a vertical sounding rocket were used, and it was found that some of the regions of the sky that appear bright when viewed in this ultra-violet light do not appear so when viewed from the ground in visible light; this is a portent of new discoveries to come. It is important to note that the exploitation of these possibilities does not require the use of deep-space probes, as a satellite circulating a few hundred miles up will be quite adequately outside the atmosphere (see figure 7). More elaborate equipment, such as an instrument platform automatically stabilized in a plane effectively fixed with respect to the stars, will be an essential requirement as the subject develops; but this does not represent too serious a technical problem.

METEOROLOGICAL APPLICATIONS

So far we have been concerned with observations looking outwards from the Earth, but much of value can be obtained by looking inwards at the lower atmosphere with equipment carried in an artificial satellite. In this way the cloud cover over the whole Earth can be under continuous observation and features undetected from the ground may be clearly discerned (figures 3, 4, 6). These include the development of tropical storms and other disturbances; information of this kind cannot fail to assist meteorologists in day to day weather prediction. Moreover, in association with regular observations of the gain and loss of heat from the atmosphere, which can also be made with satellite-borne equipment, it may even lead to much deeper knowledge and understanding of atmospheric circulation, so making long-range weather forecasting possible.

LUNAR PHYSICS

The Moon is an especially interesting object to

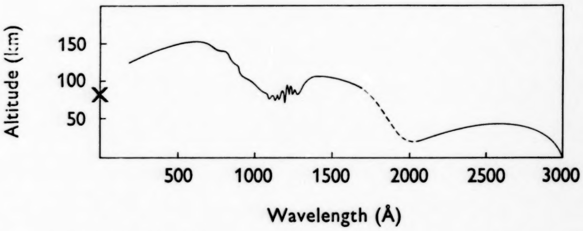


FIGURE 7 – *Height in the atmosphere at which ultra-violet- and X-radiation entering from outside is reduced in intensity to* 1/2·7 *of its initial value. The corresponding height for X-rays is indicated by* **X**.

study because of the light that may be thrown on the origin of the solar system. The thermal history of the Moon—whether it has ever been molten—is of particular interest. In this context, the measurement of lunar radioactivity and magnetism, if any; the determination of the chemical composition of surface material; verification of the presence or otherwise of volatile constituents; and detailed investigation of the nature of the surface are all likely to prove of much value, besides being of interest in themselves. Thus selenology would be expected to involve quite different considerations from those of geology because, apart from anything else, there has been neither erosion by water nor production of sedimentary rocks on the Moon. Moreover the exposure of the surface to meteor bombardment and short-wave radiations, as well as to the effects of extreme changes of temperature, will have introduced features unknown on the Earth.

In the first instance, lunar observations will be made from vehicles that do not land—a task begun with the Russian Luniks and the American Pioneer probes. Already there is evidence that the lunar magnetic field is very weak; but so far, except for the Russian lunar impact, the probes have not approached closer than a few thousand miles from the Moon. Before long a vehicle will be placed in a near orbit, in which it will make a considerable number of revolutions round the Moon.

From accurate observation of the orbits of such lunar satellites, new information about the shape and composition of the Moon will be forthcoming. If the orbit is sufficiently close (within about 100 km) it will be possible to investigate the spectrum of the γ-rays emitted from the Moon. This will indicate the composition of the radioactive sources responsible, which will in turn give information about the nature of the surface rocks. Tests for the presence of a thin surface-atmosphere or ionosphere, and magnetic measurements, could also be carried out from a close lunar satellite. Echo-sounding would be employed to give further information about the nature of the surface—a matter important for future landing of equipment. Television pictures of the surface would naturally play a vital part throughout.

The next stage would involve a fairly hard, but not too hard, landing of equipment. It should be possible to design a robust seismograph which would survive the initial shock and transmit back to Earth data about the occurrence and scale of moonquakes, if any. With development of technique, so that landing shocks were greatly reduced, more elaborate apparatus could be set in operation. For example, an X-ray analysis of the surface could be carried out by bombarding it with an electron beam and observing the fluorescent X-ray spectrum emitted. This is a mere foretaste of possibilities, the scope of which is only gradually being appreciated through the increased attention being paid to the subject.

PLANETARY STUDIES

Many of the remarks made about the Moon apply to the planets, of which only Mars and Venus are likely to be in range for many years to come. However, there is an important difference from the Moon, in that both possess extensive atmospheres that would greatly complicate the task of landing equipment safely on the surface: heating due to friction of the fast-moving vehicle with the atmosphere would be severe. For a considerable time attention would therefore have to be concentrated on studies of the atmosphere and ionosphere and of surface conditions, with probes circulating a few hundred kilometres from the surface.

Because of the biological information that might be forthcoming, especial importance attaches to the soft landing of equipment. The question of whether there exists, in an extra-terrestrial environment, anything that could be called living is of supreme interest in relation to the wider problem of the nature and origin of life. There is evidence suggesting the presence of some form of lower plant life on Mars, so that there are real prospects of major discoveries when information can be received about the nature of the surrounding material from instruments placed on the Martian surface. Venus may turn out to be even more surprising, as we know little about the surface, or even about the atmosphere, of this planet.

OTHER SCIENTIFIC APPLICATIONS

Although the variety and scope of the scientific work already outlined is very great, it is by no means comprehensive. There are other less obvious possibilities, such as the study of the nature of gravitation. This force is the weakest of the four major types of natural force known to us and is apparent in everyday life only because of the ubiquitous presence of a very large body, the Earth; the other three forces can be observed even between fundamental particles with masses of 10^{-24} g or less. Because it is so weak we know less about the nature of gravitation than about the other three types of force; not that our knowledge of these is very profound. With space probes available there will be opportunities for precise observations, under controlled conditions, of small orbital perturbations and of the relative rates of clocks in different gravitational environments. The prospects in these directions, though vague at present, may well turn out to be of basic scientific importance.

The most important new results that will be obtained through space research will be unexpected ones. All that has been done here by way of illustration is to list the broad headings within which investigations will proceed and some of the ways in which they will begin. As the work expands, more and more ingenious proposals will be made and implemented.

ATMOSPHERIC AND COSMIC EXPERIMENTS

So far we have been describing what types of scientific observation may be made. Hitherto all upper atmospheric and astronomical research has had to be of an observational kind, and much future work, including that carried out from space vehicles, will continue to be so. On the other hand, it is now possible to carry out experimental investigations in which conditions are deliberately modified in some controlled way and the consequences studied.

Thus the upper atmosphere may be used as a vast photochemical laboratory, free from surface effects. Various chosen materials may be ejected into the surrounding air at suitable heights, producing optical and electrical effects that can be observed from the ground (see figure 5). Thus a few pounds of sodium vapour ejected at twilight at a height of about 100 km produces a yellow fluorescent glow that lasts up to thirty minutes; a similar weight of nitric oxide introduced at about 120 km reacts, photochemically, quite vigorously with the atomic oxygen; and so on. In daytime, artificial ionized clouds may be similarly produced. Already a good beginning has been made in carrying out this kind of atmospheric experiment.

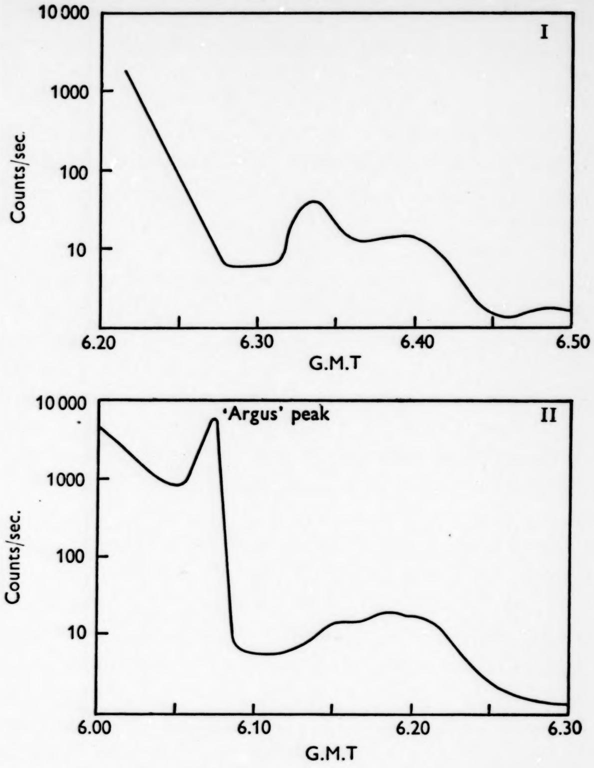


FIGURE 8 – *Results of observation of the radiation belt produced artificially in the first 'Argus' experiment. I and II show the variation with time of the counting rate of the unshielded counter in the Explorer IV satellite. I refers to rates found on 26th August 1958, the day before the experiment; II to the corresponding rates the next day, about $3\frac{1}{2}$ hours after the explosion. The peak due to the particles released from this explosion is indicated.*

By far the most remarkable large-scale experiment was successfully carried out by the United States in 1958, the 'Argus' experiment. On three separate occasions, belts of particle radiation having an intensity comparable with that of the natural belts were produced artificially by exploding atom bombs of conventional type at an approximate altitude of 480 km above a point in the South Atlantic. The charged particles produced in these explosions spread round the Earth in a belt 40–100 km thick. These belts lasted for some days, during which they were studied with equipment aboard an American satellite (see figure 8). Besides the radiation belts, artificial auroral displays were observed both above the firing site and at the conjugate[1] magnetic location in the northern hemisphere (the Azores). This was an experiment on the cosmic scale and plainly indicates what immense possibilities are now opening before us.

COMMERCIAL APPLICATIONS

The last remarks apply with equal force to any commercial applications. It is too early to predict most of these, although the practicability of large-scale modification of the Earth's environment has already been demonstrated by the Argus experiments. Furthermore, it is already clear that there are important applications of satellites to world-wide communications.

---

[1] The conjugate point is that at which the line of magnetic force through the firing point returns to the atmosphere in the other hemisphere.

At present long-distance communication by radio depends on the ability of the ionosphere to prevent the radio waves from passing tangentially out into space, but the ionosphere is effective in this way only for waves that are longer than 15 m. Shorter waves, such as are used in television, cannot, therefore, be used for long-distance transmission. Apart from this there are other limitations: the ionosphere is highly variable, particularly at high latitudes, so that communication is capricious. For these reasons it would be advantageous to replace the ionosphere by something more reliable and less dependent on the use of a particular wavelength.

One way of doing this would be to set up a number of satellites that would act as reflectors of radio waves. Such a passive system would have attendant disadvantages, many of which could be overcome by using active satellites that would receive signals and retransmit them back to the Earth. These satellites would be in such orbits that they remained fixed with respect to points on the ground. It would be necessary to include devices that could be operated on command to restore them to their correct positions from time to time as they drifted away as a result of various perturbations. However, it seems possible that a network of this kind would be a good commercial proposition, even allowing for regular replacement launchings. A further possibility is to lay down an artificial ionosphere of small conducting needles that would form a belt round the Earth at an appropriate altitude. By choosing the size of the needles this belt could form a reflecting layer for short radio-waves in a narrow wavelength band; the total weight of material required would not be prohibitive. This suggestion is, however, looked on with horror by radioastronomers, who fear that their opportunities to observe the relatively very weak signals from extra-terrestrial sources will be restricted even more by disturbances due to artificial reflecting belts of this type. But all this serves to show what big, and sometimes dangerous, possibilities can now arise.

CONCLUSION

Space research covers an extremely wide field of scientific endeavour, and its importance in the context of pure science is very great. For its success, it is necessary to develop and improve continually a wide range of techniques, including rocket propulsion, power sources, communication and other forms of electronic engineering, high-speed computation, and automation generally. The instruments required must be especially small in volume and weight, robust, and capable of operating reliably over long periods without maintenance. It is obvious that the by-products of these varied technical developments will be of much importance and value in many other fields. Apart from this, there are already forseeable practical applications, as in the field of communications, and there will undoubtedly be many more as the work develops.

Expenditure on space research is therefore likely to be a sound investment provided it is maintained within appropriate limits. This kind of research is expensive and potentially unlimited, but it is not necessary to embark on all types of activity in the field in order to obtain some dividends; for any individual country the problem is to decide how far it should go. This is obviously very difficult in any particular case, and it is clearly desirable that the maximum amount of international co-operation should be encouraged. Because of the great expense involved in providing the basic tools, space research must be highly organized; but it is essential that the organization should not be so cramping that there would be no opportunity for individual enterprise, so important in all scientific research.

ACKNOWLEDGMENT

I am particularly grateful to Dr Frutkin of the National Aeronautic and Space Agency, U.S.A., for providing the photographs taken from Tiros I.

Fluorescent protein tracing and the fluorescent antibody method

R. C. NAIRN

Fluorescent dyes may be used for labelling proteins without materially affecting their biological or immunological properties. This is most valuable for biological research, for such labelled proteins can be injected into animals and traced directly in histological sections by ultraviolet fluorescence microscopy. Alternatively, in immunological tracing, labelled serum-antibody is used as a specific histochemical stain to locate the corresponding antigen in microscopical preparations: this article describes and illustrates the study in this way of a wide variety of antigens, including micro-organisms and protein constituents of tissues.

There can be no doubt about the value to biology of a method that permits the tracing of native or foreign proteins in the tissues of living organisms. Some proteins can be labelled and traced directly, others by the application of immunological principles in which they are located histologically by means of labelled specific antibody. Such labels are provided by certain fluorescent dyes which may be combined chemically with proteins, including serum antibodies, without material effect on the biological or immunological properties of the proteins. Fluorescein [4] and lissamine rhodamine B (RB200) [2] are the fluorochromes in commonest use at present, giving respectively conjugates with apple-green and orange fluorescence in ultraviolet light. In suitable preparations of tissues, cells, and micro-organisms, these conjugates can be made visible by ultraviolet fluorescence microscopy, with considerable histological precision.

DIRECT TRACING

The fluorescent conjugates of serum proteins, when injected into animals, seem in all important respects to be accepted biologically as normal proteins [26]. They are not toxic, and their distribution in the body and eventual elimination are much the same as with proteins labelled with radioisotopes. The distribution of the proteins can be demonstrated by fluorescence microscopy in histological preparations of tissues removed from the organism, or sometimes *in vivo* as in microcirculatory studies of mesenteric loops. Fluorescent tracing is a complementary technique to radioactive tracing: the sensitivity is substantially less but this is fully compensated by its greater convenience, speed, and histological precision.

IMMUNOLOGICAL TRACING

This is an immuno-histochemical technique quite distinct from direct tracing and much more widely used. It depends on the fact that serum antibody that has been labelled with fluorescein or RB200 still retains much of its immunological activity, usually about 50 per cent. Such fluorescent antibody can be used as a specific immunological stain for micro-organisms, proteins, and other macromolecules, which can therefore be identified even in the presence of closely related organisms or substances. The principles of the method are illustrated by an experiment in which an antigen, such as a suspension of micro-organisms, is injected into a rabbit to stimulate antibody production. The antibody, formed after two or three injections over a few weeks, is present in the γ-globulin fraction of the serum which is conjugated with the fluorochrome. The conjugate and the corresponding antigen react with immunological specificity; the organisms, coated with fluorescent antibody, fluoresce brilliantly when illuminated by ultraviolet or ultraviolet-blue light. The fluorescence microscope used for this work is so designed that the light from the ultraviolet source is filtered off from the observer's eye, usually by means of a filter built into the ocular, and only the fluorescence of the specimen under examination is seen. Consequently, the apple-green fluorescence of fluorescein conjugates or the orange of RB200 conjugates is seen against a black background.

In theory, the method could be employed for visually identifying any substance capable of stimulating antibody production. In practice, two difficulties may arise. Firstly, the antigen may fail to provoke an adequate antibody response, or the presence of impurities in the antigen may give

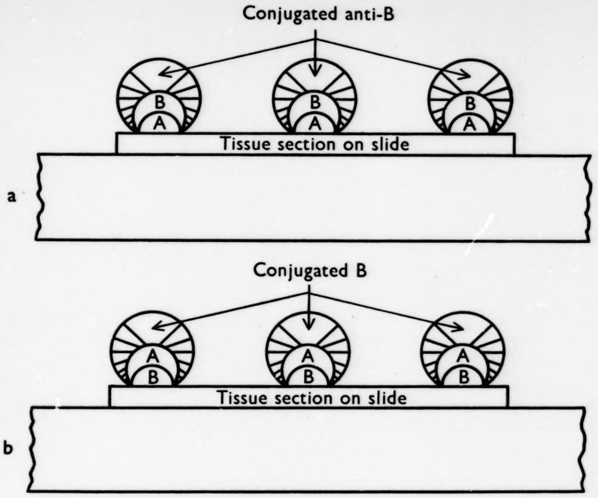


FIGURE 1 – *Double-layer (sandwich) methods in fluorescent antibody tracing.* (a) *Location of antigen (A) with layer of unconjugated specific antiserum (B) followed by a layer of conjugated antiglobulin. If, for example, B is rabbit antiserum, then the second layer would be anti-rabbit-globulin prepared in some other animal.* (b) *Location of antibody (B) by applying a layer of specific antigen (A) which is then made evident by a layer of conjugated B antiserum.*

rise to unwanted antibodies. Secondly, there may be inability to fix and preserve the antigen in preparations suitable for microscopy. These difficulties can often be overcome, and there is a growing literature about the applications and technical development of the fluorescent antibody method. The antigens that have been studied include tissue components such as connective-tissue elements, cytoplasmic and nuclear proteins, enzymes, and hormones, and also helminth parasites, protozoa, fungi, bacteria, and viruses. The localization of antigen is carried out in fresh-frozen or freeze-dried tissue sections, in smears, or in tissue culture preparations. For insoluble antigens such as tissue reticulin or collagen, preparations may be treated directly with the fluorescent antibody; soluble antigens require initial fixation with some histological fixative such as acetone or 95 per cent ethanol, which rarely destroys antigenicity. Conventional fixatives and dehydrating methods are avoided because they usually denature the antigens.

An alternative way of applying the antibody technique is by the double layer or 'sandwich' method (figure 1*a*). The antigen (A) is first coated with unlabelled specific antibody globulin (B) which in turn is located with a labelled antiglobulin serum. This method has the advantage of greater sensitivity than the single-layer method and it reduces the number of conjugated sera required for work with several different antigens. For example, a single antiserum, prepared against rabbit globulin in an animal such as a goat, can be conjugated and used to detect any rabbit antiserum attached to its corresponding antigen. A different kind of sandwich technique may be used to locate antibody in animal tissues (figure 1*b*). In this, the preparation is treated with the corresponding antigen (A) which becomes attached to the antibody (B) in the tissues and can then be located with labelled antibody.

TECHNIQUE

For direct tracing, the conjugated serum protein is used at the same protein concentration as serum and injected intravenously in a dose of about 8 ml/kg body weight. The tracer is easily identifiable histologically by fluorescence microscopy up to six hours after injection [21].

For immunological tracing, conjugated antiserum is used and the first step is commonly the preparation of the specific antibody. The antigen to stimulate antibody production should be as pure as possible in order to avoid the production of unwanted antibodies; it may be injected in saline solution into suitable animals, but frequently is first mixed with an adjuvant, such as Freund's. Adjuvants retard the absorption of the antigen and also promote, by non-specific inflammatory reaction, the production of antibody-forming cells in the immunized animal. Usually two or three injections of about 5–50 mg of antigen per kilogram body weight at monthly intervals give a satisfactory antibody response, which may be measured by conventional *in vitro* tests such as agglutination, precipitation, or complement fixation. The antiserum, which often contains as much as 3 mg of antibody globulin per millilitre, is conjugated with the fluorochrome; the conjugate is treated with ammonium sulphate (40 per cent saturation) to precipitate the γ-globulin and thus concentrate the fluorescent antibody, which is then resuspended in physiological saline and dialysed to remove the ammonium sulphate.

Chemical combination of serum protein with the fluorochrome is believed to take place largely through the free amino group of the lysine moieties. For this combination to take place, the fluorochrome molecule must contain a reactive group capable of forming a stable covalent linkage with the free amino-group. Fluorescein is used as the isocyanate or isothiocyanate [25], which form carbamido and thiocarbamido linkages respectively; RB200 is used as the sulphonyl chloride, FIGURES 2 – 7 *are fluorescence photomicrographs obtained by using ultra-violet-blue illumination with a yellow filter above the object; autofluorescence is greenish with this optical system.*

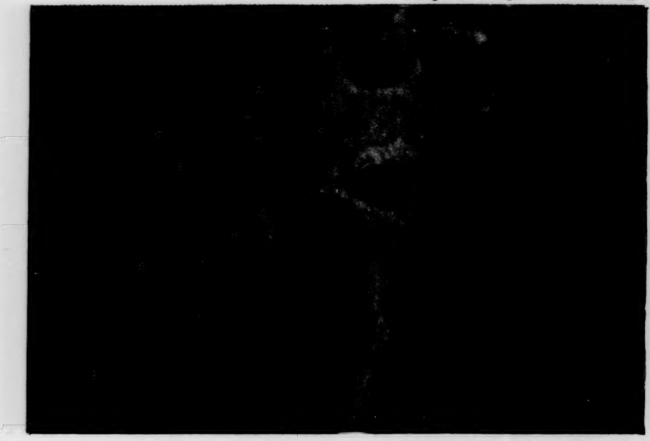


FIGURE 2 – *Influenza A virus in syncitial clump of calf testis cells stained with RB200 anti-influenza globulin. Specific fluorescence in cytoplasm but no staining of nuclei. Faint greenish fluorescence, left of infected clump, is an uninfected cell.* (× 800)

FIGURE 3 – *Organ-specific antigen in human epidermis to the right, with absence from underlying cancer to the left. Section of skin treated with anti-human-skin serum. Specific fluorescence is conspicuous in cell membranes.* (× 550)

FIGURE 4 – *Combined klebsiella and pneumococcal infection in mouse. Peritoneal smear treated successively with RB200 anti-klebsiella globulin and fluorescein anti-pneumococcal globulin. Klebsiella stained orange and pneumococci green. Three greenish mesothelial cells left of centre.* (× 800)

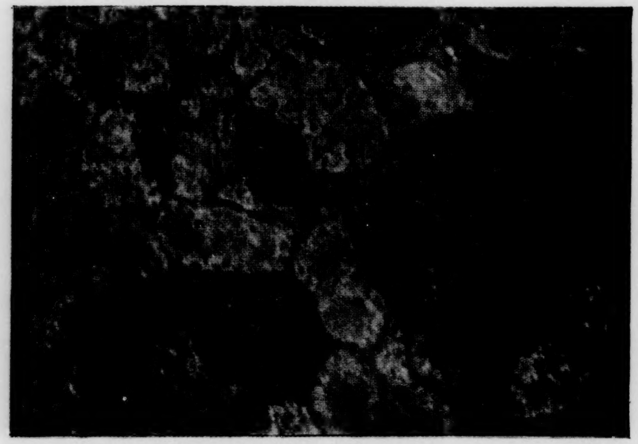


FIGURE 5 – *Renin in pig kidney. Section treated with anti-renin serum and then RB200 goat anti-rabbit globulin (sandwich method). Specific fluorescence in cells of the glomerula tuft and capsule; autofluorescence in the tubules.* (× 180)

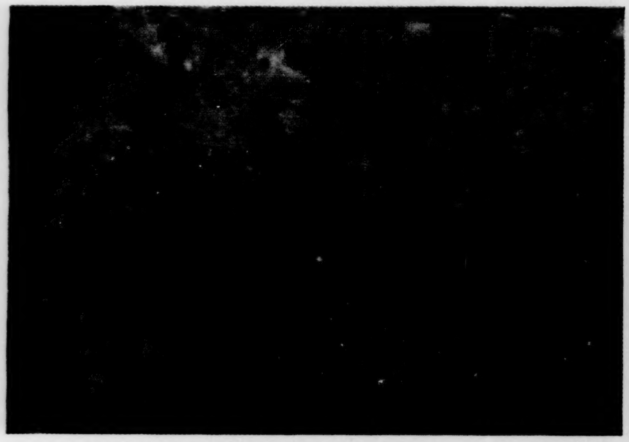


FIGURE 6 – *Demonstration of anti-nuclear antibodies in serum from a man with disseminated lupus erythematosus, an 'auto-immune' disease. Section of rat liver treated with the patient's serum conjugated with RB200. Specific nuclear fluorescence against greenish autofluorescent background; quadrantal space, top left, is a centrilobular vein.* (× 550)

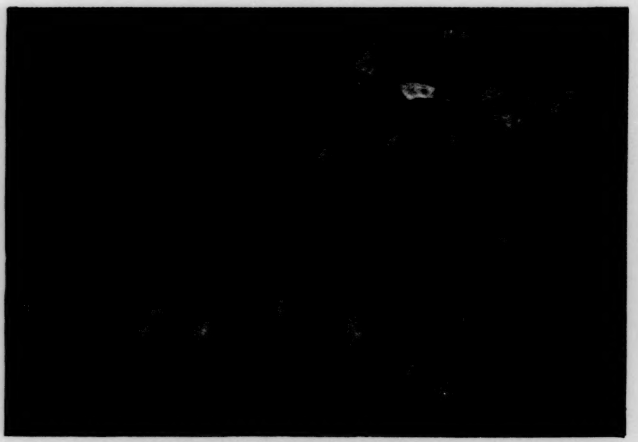


FIGURE 7 – *Section of normal lung from a rat which had been injected intravenously with normal rat serum conjugated with RB200. The orange fluorescent plasma contrasts well with the green autofluorescence of the lung tissue and the black non-fluorescent alveolar spaces; the small black bodies in the fluorescent plasma are red cells, which do not fluoresce.* (× 550)

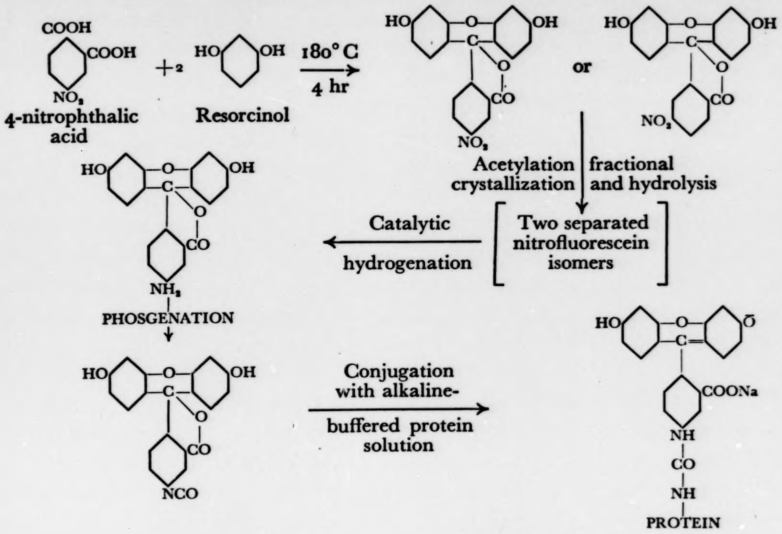

COOH
COOH
NO$_2$
4-nitrophthalic acid
+2
HO
OH
Resorcinol
180° C
4 hr
HO
OH
O
C
CO
NO$_2$
or
Acetylation
crystallization
fractional
and hydrolysis
Two separated nitrofluorescein isomers
Catalytic
hydrogenation
NH$_2$
PHOSGENATION
NCO
Conjugation with alkaline-
buffered protein solution
COONa
NH
CO
NH
PROTEIN

FIGURE 8 – *Preparation of fluorescein-conjugates. For isothiocyanate conjugation the more convenient liquid thiophosgene ($CSCl_2$) replaces the gaseous phosgene ($COCl_2$) and sulphur replaces the oxygen in the resulting reactive group.*

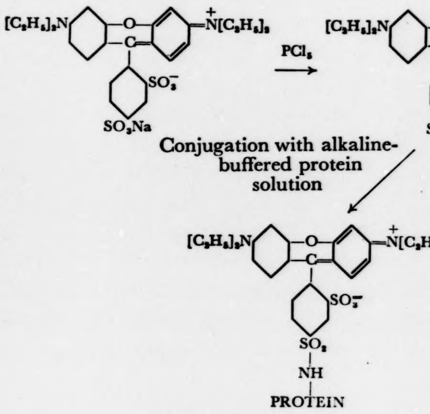

[C$_2$H$_5$]$_2$N
N[C$_2$H$_5$]$_2$
PCl$_5$
SO$_3^-$
SO$_3$Na
SO$_2$Cl
Conjugation with alkaline-
buffered protein
solution
SO$_2$
NH
PROTEIN

FIGURE 9 – *Preparation of conjugates with lissamine rhodamine B (RB200).*

which gives a sulphonamido linkage. The essential steps of the preparations are shown in figures 8 and 9. Fluorescein isothiocyanate is replacing isocyanate in practice because it is a stable substance and obtainable commercially, but it is expensive—about £10 per g compared with less than 6d. for RB200.

An excess of dye is used in the conjugation, and some of this is adsorbed by the serum proteins. Free dye in solution, and most of the adsorbed dye, may be removed by dialysis, by passage through resin columns, or by shaking with absorbing materials such as dried tissue powders; virtually all unconjugated dye can be removed by shaking with powdered activated charcoal [3].

The purpose of these absorption procedures is to obtain a fluorescent solution in which all the dye is firmly bound to the serum proteins. After centrifuging to remove particulate matter, conjugates are stored at −20° C until required. For most purposes, antiserum conjugates need further absorptions with tissue powders or homogenates to remove unwanted antibodies or other proteins which may be a source of non-specific staining reactions. The conjugates are applied to the antigen, such as a tissue section, as a drop, and the preparation is kept in a damp container for 10–60 minutes to allow the reaction to take place. The surplus conjugate is then removed by washing in buffered saline, *p*H 7·0. A glycerol mountant, which is non-fluorescent, is used and the preparation is then ready for examination by fluorescence microscopy.

The microscopy demands a powerful source of ultraviolet-blue light. For this, the modern high-pressure mercury-vapour burner has largely replaced the carbon arc; it provides a small intense source with fairly constant output of wavelength 2750–6000 Å, with main peaks at 3650 Å and 4348 Å. Filters that pass ultraviolet or ultraviolet-blue transmit light up to 4100 Å or 4750 Å respectively, a suitable range for stimulating fluorescence in the fluorochrome labels in common use. Quartz lenses are not necessary for this long-wave ultraviolet light, but the collector lens and condenser of the microscope should be made of crown glass, which transmits light above 3000 Å, and surface-reflecting mirrors should be used. Dry objectives giving up to 60 × magnification are most convenient; when using oil-immersion objectives to obtain higher magnification, a dark-ground condenser and non-fluorescent oil are required.

The fluorescent antibody method permits precise histological localization of antigen at concentrations as low as 10 μg/ml [6]. Fluorescent tracers can be seen in much lower concentration (1 μg/ml) than is possible with non-fluorescent dyes. Although this is more than is required with radioactive tracers for autoradiography, the gain in histological precision with the fluorescent method is adequate compensation. Such precision often demands special equipment to obtain good microscopical preparations: for example, a low-temperature microtome cabinet is used to obtain uniform thin frozen sections.

RESULTS ACHIEVED

The direct tracing method has so far been little used. The physiological distribution of fluorescein-conjugated serum proteins was demonstrated by A. A. Schiller, R. W. Schayer, and E. L. Hess [26] in 1953, and an application of the technique to the study of experimental liver damage, using labelling with both fluorescein and RB200, was reported in 1958 [21]. J. Oort, in a recent personal communication, has described the use of human serum albumin labelled with RB200 as a tracer in the investigation of a rare disease of childhood characterized by protein-losing diarrhoea. Figure 7 demonstrates the kind of histological picture obtained by the direct method. Fluorescent plasma is shown in the capillaries of an unstained paraffin section of normal lung from a rat that had been injected intravenously one hour before with normal rat serum conjugated with RB200.

Immunological fluorescent tracing has had many applications in biological and medical research. Its value to microbiologists and immunologists has been unquestioned since A. H. Coons and his colleagues published their important series of studies on the distribution of foreign antigens, on the natural history of virus infections, and on antibody formation [5, 6]. The simplest example of immunological tracing is perhaps the specific staining of bacteria by a conjugated antibacterial serum. The term specific staining here denotes the process of coating antigen with fluorescent antibody and of proving by suitable tests that the reaction is specific. Control tests commonly used are: (1) that fluorescent staining occurs only when the corresponding antigen is present; (2) that no staining of the antigen occurs when conjugated normal serum is used; (3) that staining is inhibited if the antigen is pretreated with unconjugated specific antiserum; (4) that staining is inhibited if the conjugated antiserum is first absorbed (neutralized *in vitro*) with the corresponding antigen.

In medical research, there are already numerous examples of the method having been used to identify a specific organism in a mixed flora—for example, Group A streptococci in throat washings [19]. The method has also been used in veterinary research for the rapid identification of bacterial flora in the rumen contents of sheep and calves [11]. Similar methods may be applied to botanical research: specific soil organisms have been demonstrated in and around plants, and their spatial distribution defined, despite the presence of large numbers of contaminating organisms.

A refinement of this kind of immunological tracing is illustrated in figure 4, in which two different organisms are demonstrated simultaneously by double staining. The photomicrograph is of a smear of peritoneal exudate from a mouse infected with both a pneumococcus and a klebsiella. The smear, after acetone-fixation, was treated with anti-klebsiella globulin conjugated with RB200 and then with fluorescein-conjugated anti-pneumococcal globulin; the two bacterial antigens are clearly visible in contrasting colours. This method has also been used to distinguish two related strains of the protozoon *Trichomonas* in mixed smears [2]: cross absorption of the conjugates by the antigens is necessary in such experiments to obtain specific staining of each strain with its own conjugate only. Double tracing has obvious application to many immunological problems; it has been used for the separate identification of different antibody-producing cells in a single lymph node [29], and for the study of antibodies to closely related antigens [27].

The remaining photomicrographs illustrate some other applications of the fluorescent antibody technique. The use of RB200-conjugates in each of these reflects the author's special interest in this tracer; fluorescein-conjugates would serve as well wherever good colour contrast with the autofluorescence of the tissue is not essential. Autofluorescence is a natural phenomenon in biological material: it is usually blue-green in animal and plant tissue, except for the green parts of plants, which exhibit the red fluorescence of chlorophyll. Fluorescein does not provide good colour contrast with the normal blue-green autofluorescence of biological material, but this may not be important if the fluorescent intensity of the tracer is sufficient. When the intensity is poor, good colour contrast is essential and is provided by RB200.

Figure 2 demonstrates the value of the method for the localization of virus. It shows specific fluorescence due to the presence of influenza virus particles in the cytoplasm of an infected tissue-culture cell. The preparation is a coverslip monolayer fixed in acetone and stained with anti-influenza A globulin labelled with RB200. Such preparations offer a method of studying the natural history of virus infection—its location and development. The cytological localization can be sufficiently precise to permit a clear distinction to be made between antigen accumulation on the cell membrane, in the cytoplasm, in perinuclear membrane, and in the nucleus. Similar location of influenza virus has been made in experimental animal tissues [8] and in human material [15]. Many viruses have been successfully investigated in this way [16] and much information has been gathered on infectivity, growth cycles, and spread. Without the fluorescent antibody method, such information would have been obtained only with difficulty or not at all.

Figure 5 is an example of the use of the technique for the location of an antigenic substance native to the tissues studied—in this case the pressor enzyme, renin, in pig kidney [22]. For this study, renin, prepared in as pure a state as practicable by chemical fractionation of pig-kidney extract, was injected with adjuvant into rabbits to provoke the formation of anti-renin serum. This was absorbed with pig-tissue preparations to remove unwanted anti-pig and anti-kidney components and to leave the anti-renin. The absorbed serum was then applied to frozen sections of pig kidney after fixation in 95 per cent ethanol; the site of its reaction with the renin was located by subsequent application of anti-rabbit globulin conjugated with RB200. Specific fluorescence was obtained in the cells of the glomerular tufts, but not if the anti-renin serum had been previously neutralized by mixing with the measured amount of renin. The findings do not demonstrate conclusively that the material located is renin, because the renin solution used for the neutralization test was contaminated by related proteins; pure renin is not yet available. Other enzymes and some hormones have been located histologically with fluorescent antibody—for example, pancreatic enzymes [17, 20], insulin [13], and pituitary growth-hormone [14]. Successfully demonstrated native antigens also include plasma proteins [9], reticulin and basement membrane [7], the blood group substances in gastric and duodenal mucosa [10], and proteins in muscle [18].

Figure 3 illustrates an interesting new development of the technique. It is a frozen section of a malignant tumour (naevocarcinoma) in human skin and shows specific staining of the normal epidermis by an anti-human-skin serum fully absorbed to remove other anti-human-tissue antibodies and leave only anti-skin activity. Here the serum has failed to stain the underlying cancer cells: by contrast, benign tumours of skin stained as strongly as the adjacent normal epidermis. Such findings support the view that malignant tumours lack specific material present in the normal tissues from which the tumours have arisen. An important concept here is that normal tissues contain antigenic components that are specific for the tissues. P. Vogt [28] has shown by fractionation of rat-liver cells that the organ-specific antigen is confined to the cytoplasmic membranes. Fluorescent antibody studies corroborate this view in the case of rat liver and other tissues, and further suggest that organ-specific antigen is distributed in surface membranes of tissue cells [23]. This observation may have a bearing on some fundamental biological phenomena, for example, the natural mutual adhesiveness of related cells; the normal organization of cells in tissue; and the 'homing effect', in which injected cells tend to settle in the organ of their origin. Such a surface self-marker might be required for recognition and control of normal cells by the body; its absence in cancer cells could be responsible for their uncontrolled malignant growth.

The demonstration of serum antibodies in man and animals is another important use of fluorescent tracing: the serum to be tested is conjugated and applied to suitable preparations of antigens against which antibodies are suspected to be present in the serum. The method permits the diagnosis of recent illnesses due to bacterial or virus infection: the suspected antigen, obtained by culture, is stained specifically by the patient's serum, which may itself be conjugated or, alternatively, located by the 'sandwich' method using conjugated anti-human globulin. This kind of application is rarely required in practice since simpler diagnostic tests are already available, but there are circumstances in which the fluorescence technique may provide information difficult to obtain in any other way. Figure 6 is an example in which human serum antibody to mammalian nuclear proteins is demonstrated by the production of specific nuclear staining in a normal tissue section. Anti-nuclear antibodies, which do not appear to have species specificity, were first demonstrated by the fluorescent antibody technique in 1957 [12]. They have aroused considerable interest because of their association with a group of human diseases of obscure aetiology, now often referred to as autoimmune diseases. It is believed by some, though by no means proven, that antibodies developed by certain people against their own tissues are responsible, at least in part, for clinical manifestations of disease; there is increasing evidence of this in a type of thyroid disease [24]. Apart from its clinical aspects, auto-immunity has important theoretical implications for fundamental immunology [1]. The questions which arise, but are not yet answered with any unanimity, are: what normally prevents animals from producing antibodies to their own cells and tissues, and why does this mechanism sometimes break down?

FUTURE OF FLUORESCENT TRACING

Direct fluorescent protein tracing has been little explored, but it seems safe to predict increasing use of it in biological research. The information it can provide is histologically precise and complementary to the more quantitative information obtainable by radioactive tracing.

Fluorescent antibody tracing is expanding rapidly from microbiological research and applied medical use, into fundamental studies of auto-immunity and of the immunological aspects of species and organ specificity, embryology, genetics, and carcinogenesis. Its increasing use in veterinary and botanical research may be confidently expected. The method is perhaps the most elegant and specific of all the histochemical techniques; its only limitations are those of immunology itself.

ACKNOWLEDGMENTS

The author has pleasure in acknowledging the help received from colleagues in the fluorescence microscopy research team, Dr J. E. Fothergill, Dr K. B. Fraser, and Dr M. G. McEntegart.

REFERENCES

[1] Burnet, F. M. *Brit. Med. J.*, **ii**, 645, 720, 1959.

[2] Chadwick, C. S., McEntegart, M. G., and Nairn, R. C. *Immunology*, **1**, 315, 1958.

[3] Chadwick, C. S. and Nairn, R. C. *Ibid.*, **3**, 363, 1960.

[4] Coons, A. H., Creech, H. G., Jones, R. N., and Berliner, E. J. *J. Immunol.*, **45**, 159, 1942.

[5] Coons, A. H. *Internat. Rev. Cytol.*, **5**, 1, 1956.

[6] *Idem. Schweiz. Z. Path. Bakt.*, **22**, 693, 1959.

[7] Cruickshank, B. and Hill, A. G. S. *J. Path. Bact.*, **66**, 283, 1953.

[8] Fraser, K. B., Nairn, R. C., McEntegart, M. G., and Chadwick, C. S. *Ibid.*, **78**, 423, 1960.

[9] Gitlin, D., Landing, B. H., and Whipple, A. *J. exp. Med.*, **97**, 163, 1953.

[10] Glynn, L. E., Holborow, E. J., and Johnson, G. D. *Lancet*, **ii**, 1083, 1957.

[11] Hobson, P. N. and Mann, S. O. *J. gen. Microbiol.*, **16**, 463, 1957.

[12] Holborow, E. J., Weir, D. M., and Johnson, G. D. *Brit. med. J.*, **ii**, 732, 1957.

[13] Lacy, P. E. *Exp. Cell. Res. Suppl.*, **7**, 296, 1959.

[14] Leznoff, A., Fishman, J., Goodfriend, L., McGarry, E., Beck, J., and Rose, B. *Proc. Soc. exp. Biol. Med.*, **104**, 232, 1960.

[15] Liu, C. *Ibid.*, **92**, 883, 1956.

[16] *Idem. Ergebn. Mikrobiol. Immunforsch.*, **33**, 242, 1959.

[17] Marshall, J. M. *Exp. Cell. Res.*, **6**, 240, 1954.

[18] Marshall, J. M., Holtzer, H., Finck, H., and Pepe, F. *Ibid., Suppl.* 7, 219, 1959.

[19] Moody, M. D., Ellis, E. C., and Updyke, E. L. *J. Bact.*, **75**, 553, 1958.

[20] Moon, H. D. and McIvor, B. C. *J. Immunol.*, **85**, 78, 1960.

[21] Nairn, R. C., Chadwick, C. S., and McEntegart, M. G. *J. Path. Bact.*, **76**, 143, 1958.

[22] Nairn, R. C., Fraser, K. B., and Chadwick, C. S. *Brit. J. exp. Path.*, **40**, 155, 1959.

[23] Nairn, R. C., Richmond, H. G., McEntegart, M. G., and Fothergill, J. E. *Brit. med. J.* **ii**, 1335, 1960.

[24] Roitt, I. M. and Doniach, D. *Brit. med. Bull.*, **16**, 152, 1960.

[25] Riggs, J. L., Seiwald, R. J., Burckhalter, J. H., Downs, C. M., and Metcalf, T. G. *Amer. J. Path.*, **34**, 1081, 1958.

[26] Schiller, A. A., Schayer, R. W., and Hess, E. L. *J. gen. Physiol.*, **36**, 489, 1953.

[27] Scott, D. G. *Immunology*, **3**, 226, 1960.

[28] Vogt, P. *Z. Naturf.*, **15b**, 221, 1960.

[29] White, R. G. *Nature, Lond.*, **182**, 1383, 1958.

Ultra-high vacuum

G. LEWIN

The phrase ultra-high vacuum has no precise definition, but is generally taken to apply to pressures less than 10^{-11} atmospheres. In recent years the attainment of vacua of this kind has become increasingly necessary, and commercial equipment has been developed. Ultra-high vacua are important in the study of photoelectric, thermionic, and field emission of electrons, and to the physicist interested in the properties of absolutely clean surfaces.

In an earlier article in ENDEAVOUR, E. N. da C. Andrade [1] has recounted the evolution of the vacuum pump, bringing the story up to the early nineteenth century, when pressures of about 20 torr could be obtained. (The torr, named in honour of Torricelli, is the unit of pressure commonly used in high-vacuum physics: 760 torr equals one atmosphere.) Today very much lower pressures are necessary. A pressure of 10^{-6} torr is typical for today's high-vacuum systems used in the production of electronic tubes. Although not precisely defined, the ultra-high vacuum region is generally taken to begin at about 10^{-8} torr, but 10^{-9} torr may be said to be more typical of modern practice.

At pressures such as these, gases are very rarefied indeed, but conditions are still some way from those in a perfect vacuum. The gas-kinetic data indicate how some of the significant properties of air change with diminishing pressure (table 1). In this table the second column shows the number of molecules per cubic centimetre. In their random motion the molecules travel on the average a certain distance (the mean free path) before they collide with another molecule: this distance is shown in the third column. It is clear that, in high vacuum and ultra-high vacuum, molecules collide with the wall rather than with each other.

The fourth column shows the number of molecules that will hit a surface of one square centimetre area each second. If we assume that this surface was initially free from gas and that each molecule that collides with the surface sticks to it, the time required to form a monomolecular layer of gas is that given in column five; a monomolecular layer of gas contains 10^{15} molecules per cm^2. It follows that ultra-high vacuum is required to retain a clean surface for a reasonable length of time. Ultra-high vacuum is therefore of paramount importance wherever surfaces have to be studied and wherever very clean surfaces are desired. Typical applications are the preparation of transistors, and of magnetic and super-conductive films by vapour deposition; and the study of photoelectric, thermionic, and field emission of electrons.

Some characteristics of gas discharges are significantly affected by impurities present at concentrations of as little as one part per million. Studies of such discharges require clean walls and low base-pressures. The need for these experimental

TABLE I

Gas-kinetic data for air

	1 Pressure (torr)	2 Molecules (cm^3)	3 Mean free path (cm)	4 Molecules incident on cm^2 per second	5 Time to form monolayer (sec)
Atmospheric pressure ..	760	$2{\cdot}6 \times 10^{19}$	7×10^{-6}	$2{\cdot}3 \times 10^{23}$	4×10^{-9}
Vacuum	1	$3{\cdot}5 \times 10^{16}$	5×10^{-3}	3×10^{20}	3×10^{-6}
High vacuum	10^{-6}	$3{\cdot}5 \times 10^{10}$	5000	3×10^{14}	3
Ultra-high vacuum	10^{-10}	$3{\cdot}5 \times 10^{6}$	5×10^{7}	3×10^{10}	$2{\cdot}88 \times 10^{4}$

conditions brought about the systematic investigation of ultra-high vacuum by D. Alpert and his collaborators twelve years ago [2].

Clean surfaces are also required in connection with machines for thermonuclear research, the operation of which is adversely affected by gas impurities, as has recently been described in ENDEAVOUR [3]. Gases used in these devices must be extremely pure, to avoid adsorption of impurities on the walls of the container and their subsequent release under the influence of the discharge.

A new field of application is in the testing of components that have to perform in outer space—such qualities as mechanical friction can be affected by the presence of gas layers on the surface. Also, with space research we need to measure the composition of the atmosphere at great altitude; obviously the equipment itself must contribute as little gas as possible. Besides these immediate applications, it is of interest to the vacuum physicist just to obtain a vacuum as extreme as possible.

MEASUREMENT OF ULTRA-HIGH VACUUM

To investigate ultra-high vacuum it is necessary to have means of measuring it. The commonly used method is the ionization gauge, initially developed by Buckley. It is built like an ordinary triode, but the potentials are very different from those commonly used in radio valves. It consists of a filament surrounded by a grid, which in turn is surrounded by a metallic cylinder. The filament is earthed, the grid has a positive potential of about 150 volts, and the anode is biased 30 volts negative. The electrons which leave the filament are collected by the grid; in transit they ionize the residual gases in the tube. The ions are repelled by the grid because of their positive charge and are collected by the plate (the anode), which is biased negative with respect to the grid. The number of ions produced at a given pressure depends on the type of gas, since the probability that an electron will ionize an atom is a function of the atomic structure.

The highest pressure that can be measured by a conventional ionization gauge is about 10^{-3} torr. If the pressure is reduced below 10^{-8} torr, the ion current remains at a minimum value corresponding to this pressure. Investigators sometimes suspected that they had a pressure considerably lower than 10^{-8} torr—for example, when they observed the emission from points in a field-emission electron microscope—but their ionization gauges nevertheless always indicated this same minimum pressure.

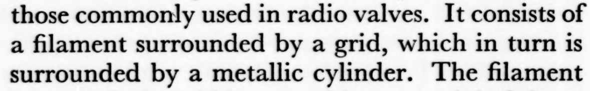


FIGURE 1 – *Bayard-Alpert inverted ionization gauge.*

The explanation of this was first given by Nottingham. The electrons that hit the grid produce soft X-rays. (This phenomenon is employed in X-ray tubes, though there the energy of the electrons, and therefore the energy of the X-rays, is 100–1000 times larger.) These X-rays release photoelectrons from the surface of the ion-collecting plate. The electron current from the ion collector to the grid is electrically indistinguishable from an ion current going to the plate, and is measured in addition to this ion current, and therefore the smallest ion current that can be measured is about equal to this photoelectric current. Its value in conventional gauges corresponds to a pressure of 10^{-8} torr.

In 1947 Bayard and Alpert introduced a gauge, now bearing their names, that reduces this photoelectric current a thousandfold. This is the inverted ionization gauge (figure 1), in which the filament is outside the grid, and the ion collector, consisting of a thin wire only, is inside it. The photoelectric current is proportional to the surface area of the ion collector, and the wire has a considerably smaller area than the cylinder in the earlier gauge. The inverted ionization gauge permits the measurement of pressures down to the 10^{-11} torr range, and is the type commonly used in ultra-high-vacuum work.

One difficulty remains. The heating effect of the hot tungsten filament releases gas from the other parts, and chemical reactions occur between the hot filament and some gases. J. P. Hobson and P. A. Redhead [4] therefore developed a cold-cathode ion gauge which permits measurements of pressures as low as 10^{-13} torr. This gauge is designed like a magnetron. The gas discharge is sustained at low pressures by lengthening the path of the electrons; this increases the probability of an electron hitting a molecule.

All ion gauges act as pumps, since the ions that hit the ion collector or the wall will be buried. This effect is made use of in the so-called ion pumps, which will be discussed later.

Recently, mass spectrometers have been increasingly replacing ion gauges. Mass spectrometers measure the number of atoms of any particular mass present in a given volume of gas; this means that in effect they measure the partial pressure of each gas. Since, as will be shown later, the composition of the residual gas left in an evacuated system has very little resemblance to that of the gas originally present, it is very desirable to determine not only the total pressure but the composition of the gas. Mass spectrometers have no X-ray limitation and they can be built with a high sensitivity, measuring partial pressures as low as 10^{-12} torr. Their disadvantage is that they are considerably more complicated and more expensive than ion gauges.

Mass spectrometers are made in a number of different forms. In one common design, ions are produced in the same manner as in the ion gauge or by a cold-cathode discharge. These ions are accelerated in an electric field and deflected by a magnetic field. For each value of field strength, only ions having a particular ratio of charge to mass can reach the collector, which is located behind a narrow slit.

THE PROCESS OF EVACUATION AND THE DESIGN OF VACUUM SYSTEMS

It is customary to measure the pump action in terms of a pumping speed, *S*, expressed in litres/sec: *S* is the volume of gas removed from the container each second at the pressure existing at the time. The reason for this definition is that the pumping speed of most pumps is approximately constant over a large range of pressures. The total mass of gas removed each second is called the throughput of the pump and is expressed in torr litres/sec: it decreases as the pressure drops.

If a pump is connected to a container an equilibrium will be reached eventually, as is shown schematically in figure 2. At the equilibrium pressure *P* the pump removes the amount of gas *SP* each second; this is equal to the total influx of gas, which comes from three sources. Q_P is the gas coming from the pump and the pump line, that is, gas desorbed from the wall of the pump line; Q_W is the gas desorbed from the walls of the container; and Q_L is gas leaking into the container from the outside. Q_P and Q_W may decrease with time, but only slowly. In ultra-high-vacuum systems Q_P is often the largest source of gas; in other words, the ultimate vacuum is limited by the performance of the pumping system. The gas initially filling the volume is removed and the gas eventually present differs from that originally present.

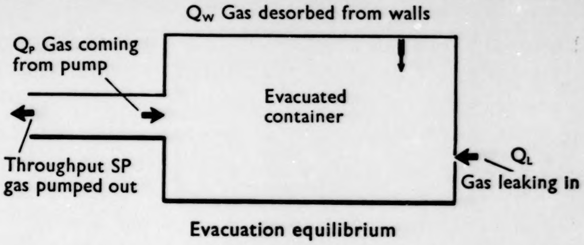

Q_W Gas desorbed from walls
Q_P Gas coming from pump
Evacuated container
Throughput SP gas pumped out
Q_L
Gas leaking in
Evacuation equilibrium

FIGURE 2 – *Schematic representation of factors affecting equilibrium in an evacuated container.*

Factors affecting Q_P will be discussed in conjunction with pumps. Q_L represents the leaks in the system; though often quite annoying, they can be avoided by proper techniques and the use of a sensitive mass-spectrometer leak-detector. Q_W is the gas given off by the walls, and the development of methods of reducing this gassing rate was the most important step in the attainment of ultra-high vacuum. Gas absorbed in the bulk of a wall, metallic or otherwise, will diffuse to the surface when the pressure is lowered. Metals generally contain a volume of gas which, if reduced to normal temperature and pressure, approximately equals the volume of the metal: this is a considerable quantity. Desorption of this gas by diffusion is very small at room temperature, but it increases rapidly as the temperature is raised. In addition, gas is adsorbed at the surface, by either physical or chemical adsorption. Physical adsorption indicates a rather weak bond between the gas molecules and the surface due to van der Waals forces and having a binding energy of about 1–6 kcal/mol. Physically adsorbed molecules can be desorbed by baking at a few hundred degrees. Chemical adsorption corresponds to a much stronger bond, similar to the ordinary chemical bond. Baking at a few hundred degrees still leaves at least a monolayer of chemically adsorbed molecules.

Although much work has been done, the evolution of gas by desorption is not sufficiently understood to make it possible to predict the behaviour of materials in ultra-high vacuum. Usually only specific examples of outgassing have been published, and from these it is not possible to formulate generally applicable laws. During evacuation, metal and glass surfaces give off water vapour corresponding to many monolayers, besides some carbon monoxide and dioxide. Their initial gassing rate is about 10^{-8} torr litre/cm^2 sec, but this gradually decreases. H. A. Steinherz [5] showed experimentally, and illustrated by calculations, that, with large stainless steel containers of over 1000 litres capacity, a pressure of 10^{-8} torr can be reached after pumping for one day; if the container is baked for five hours at 260° C, however, a pressure of 2×10^{-9} torr is obtained after a total time of only 12 hours. Baking is thus very effective in reducing the gassing rate. The gassing rate of glass and stainless steel will decrease by a factor of 10^7 (to 10^{-15} torr litre/cm^2 sec) by baking at 400° C for 10–20 hours, and this is the usual method for obtaining ultra-high vacuum. Materials that withstand temperatures up to 500° C and have low vapour pressures are therefore used in the construction of the vacuum container. In practice this means glass, metals, and ceramics; organic materials are avoided. Even if the system is not baked, these inorganic materials are preferred because they have lower gas-evolution rates and lower vapour pressures.

Although the value of baking is obvious, to achieve it with complete vacuum systems required a considerable extension of vacuum technology. Alpert designed a small bakable valve made completely of metal; other metal valves have since been developed, and described in the literature. In one design, closure is obtained by pressing a copper nose against the sharp corner of a seat made of stainless steel. The copper flows, and the seat forms an intimate contact with the copper. Figure 3 shows the seat assembly of a valve 20 cm in diameter, designed by J. T. Mark [6]. It employs the 'broaching' principle: the corner of the stainless steel seat shaves off the surface of a copper cylinder and forms its mating surface.

Another important piece of equipment is a demountable joint. It is common in vacuum technique to make joints with 'O' rings that form elastic gaskets between two metal flanges. Most 'O' rings consist of synthetic rubber, but since organic materials have to be avoided in ultra-high-vacuum work, metal gaskets (usually aluminium, copper, or gold) have to be substituted. Several designs have been proposed; figure 4 shows the 'corner gold seal'. In this the gasket is a small gold wire located in a corner between two flanges. The cost of the gold wire is not excessive, since the gold in used gaskets can be recovered.

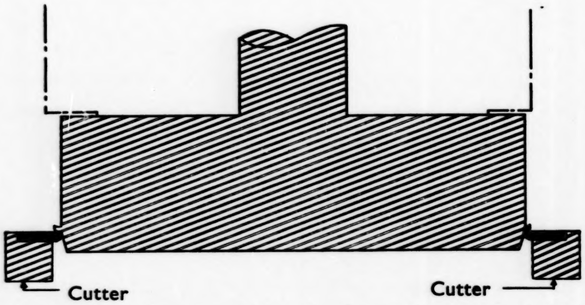


FIGURE 3 – *Seat assembly of valve designed by J. T. Mark* [6].

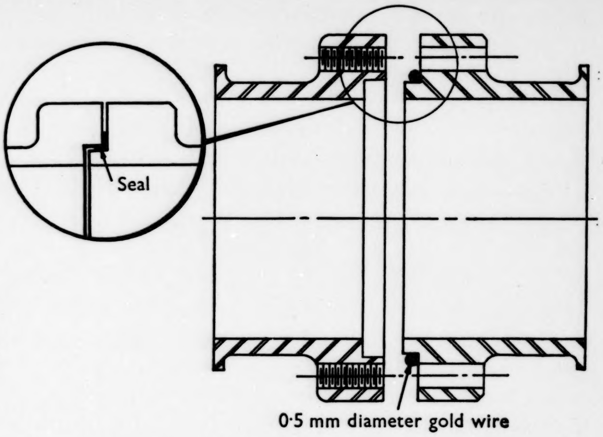


FIGURE 4 – *Ultra-high-vacuum joint with gold-wire gasket.*

Ultra-high-vacuum techniques applied to thermonuclear research have been described by D. J. Grove [7], and numerous literature references can be found in an article by G. L. Mundy [8]. Figure 5 shows an ultra-high vacuum system being tested; the pump box and pump line are seen in the left foreground. The system consists of a 20 cm diameter stainless steel pipe, in the shape of a race track, used in thermonuclear research. It is suspended in such a manner as to permit thermal expansion during baking. The parts are heated by insulating blankets containing built-in heater wires (not shown). The sections are connected by demountable flanges of the type described above.

PUMPING SYSTEMS

So far we have discussed the pumping of ultra-high-vacuum systems without saying what kind of pumps are actually used and what the limitations of these pumps are in terms of gas coming back from them. It must be remembered that the whole pumping system usually consists of a combination of several pumps. Until recently the pump system almost exclusively used consisted of a mechanical fore-pump, a diffusion pump, and a vapour trap, the three being connected in series. All these components had been employed for many years.

The fore-pump contains a rotary piston eccentrically located in a housing and running in an oil bath. Air is sucked in from one side and pushed over to the exhaust side; numerous designs are available. These pumps will pump down from atmospheric pressure to about 10^{-3} or 10^{-4} torr.

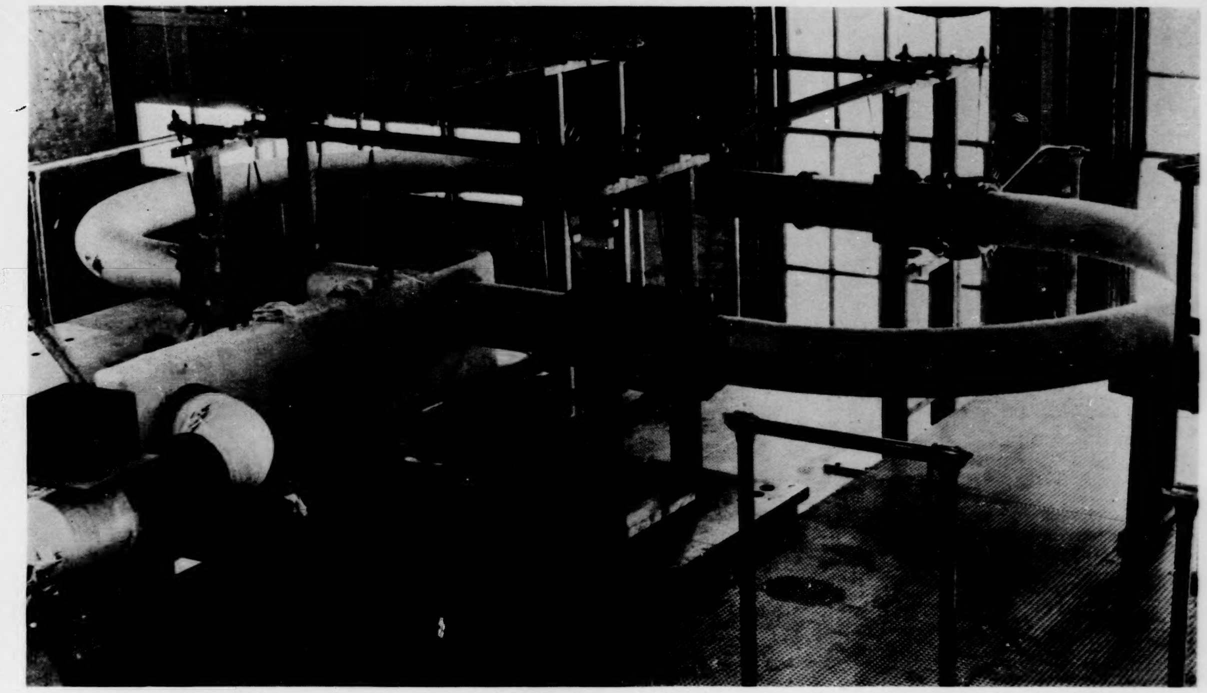


FIGURE 5 – *Ultra-high-vacuum apparatus required for thermonuclear research. The stainless steel tube of which it is constructed is* 20 *cm in diameter. The pumping apparatus is illustrated in figure* 8.

The lower pressure will be reached only with fresh oil and with two units connected in series.

The diffusion pump contains a liquid, either mercury or a special oil, that is evaporated from a boiler and streams out of a jet to hit the cooled wall of the pump (figure 6). It condenses at the wall and flows back to the boiler. The vapour leaving the jet collides with the gas molecules and gives them an additional momentum in the direction of the fore-vacuum. A pressure difference is thus produced between the space above the jet, that is, the high-vacuum side, and the fore-vacuum. These diffusion pumps can be built in large sizes having pumping speeds of thousands of litres per second.

Since some of the vapour of the pump fluid will be carried over into the high-vacuum side, it is essential to have a trap between this pump and the vacuum system. Without the trap, the limiting pressure would be the vapour pressure of the pump fluid at room temperature, which is 10^{-3} torr for mercury and about 5×10^{-8} torr for diffusion-pump oil. Two types of trap are commonly used; cold traps and adsorption traps. The cold trap shown in figure 7 puts a cold wall in the path of the vapour molecules, and on this the molecules will be condensed. These traps are cooled with liquid nitrogen to − 196° C, at which temperature the vapour pressures of mercury, pump oils, and water are extremely small. The trap shown in the picture, which is of a design suggested by Post, has the additional advantage of forming a cold-creep barrier—essential in the case of oil-diffusion pumps—that prevents the oil from creeping along the wall beyond the trap. Such a barrier is not necessary in the case of mercury, because this does not wet the walls and consequently does not creep along them. A cold trap acts as a pump for condensable vapours.

Any clean surface acts as an adsorption trap; the action of activated charcoal, for example, is

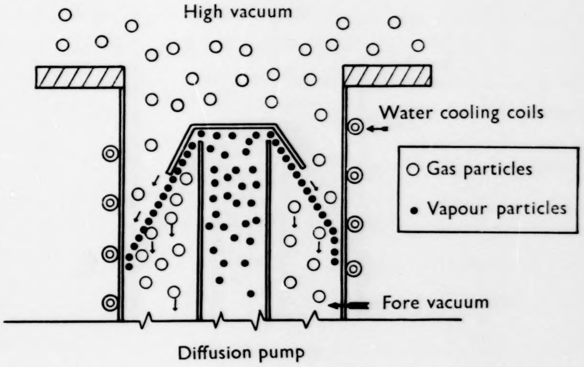


FIGURE 6 – *Schematic representation of mode of action of diffusion pump.*

well known. For ultra-high-vacuum use it must be possible easily to degas the adsorbent. This means that porous bodies, which are the first choice as adsorbers because of their large surface area, must not have internal surfaces that are connected to the outside through small pores only.

No good adsorbent is known for mercury vapour. However, Alpert found that copper foil adsorbs oil vapour, and he built laboratory systems containing a glass oil-diffusion pump with a small copper foil trap (figure 9). After baking the trap and the vacuum system, a pressure of the order of 10^{-10} torr can be maintained for over a month. When the trap becomes saturated, the pressure rises to the vapour pressure of the oil at room temperature, and re-baking is necessary. Biondi showed that traps containing zeolite or activated alumina as adsorbents have a much larger capacity and can also be readily degassed.

Pump oil decomposes to some extent during the heating process. Of the products, carbon monoxide is not removed by a cold trap, but it can be removed by an adsorption trap. Nevertheless, the ultimate pressure obtained in a diffusion pump system with an adsorption trap is not lower than that of a similar system using a cold trap: this pressure is about 10^{-10} torr for mercury and oil pumps alike. Most measurements were made with the Bayard-Alpert ionization gauge, and it appears that this gauge becomes unreliable at this low pressure. With either pump, the remaining gases are mainly carbon monoxide, methane, and helium; the latter diffuses into the system from the outside atmosphere through the glass walls.

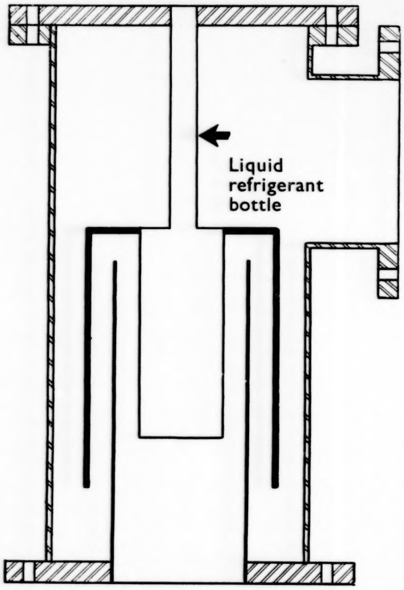


FIGURE 7 – *Trap, cooled with liquid nitrogen, incorporating barrier to prevent creep of oil from the diffusion pump.*

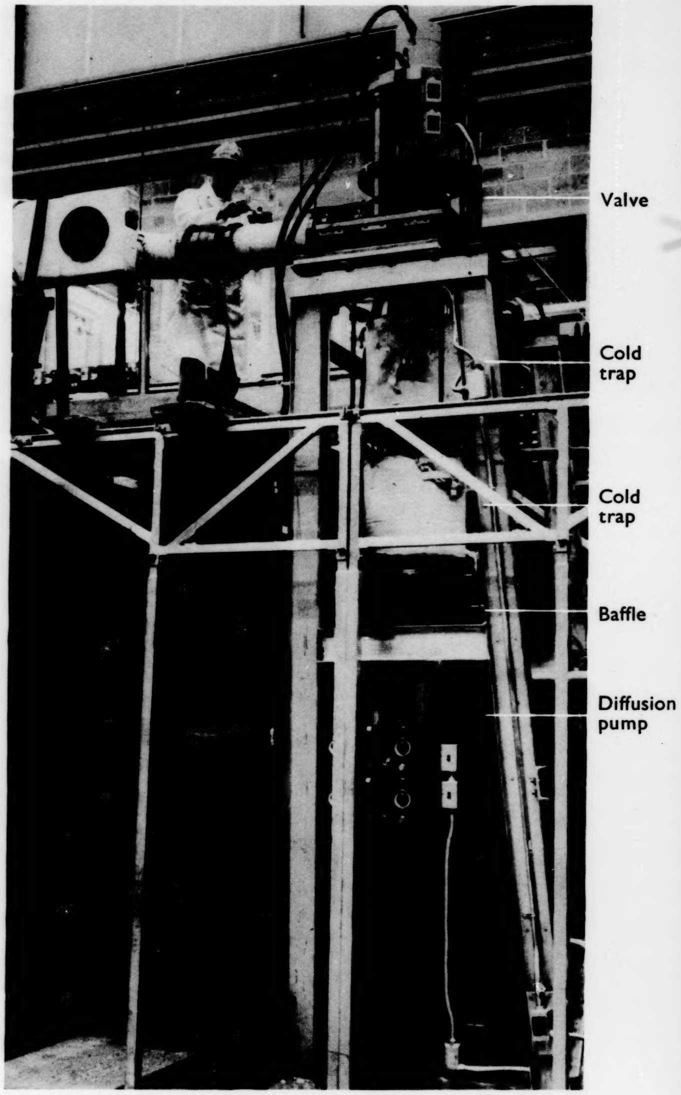


FIGURE 8 – *Pumping apparatus for ultra-high-vacuum system required in thermonuclear research.* (*See also figure* 5.)

Figure 8 shows the pumping apparatus for the vacuum system illustrated in figure 5; the mercury diffusion pump, having a diameter of 25 cm, is at the bottom (the fore-pump is not visible). Above the pump is a refrigerated baffle and two liquid nitrogen traps, which condense the mercury diffusing into the high vacuum, and the bakable valve described previously. The pumping speed is 500 litres/sec. This pumping system maintains a pressure in the 10^{-10} torr range.

The 'pumping' of gas by a film of evaporated metal is used extensively to improve the vacuum

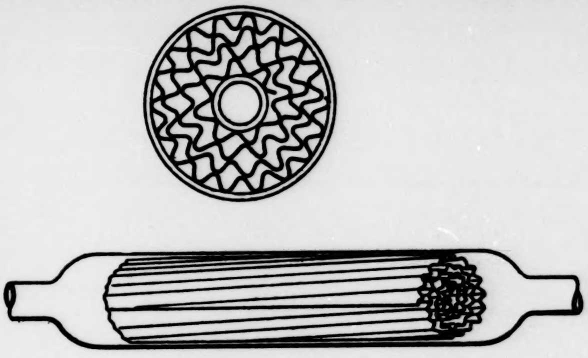


FIGURE 9 – *Copper foil trap for adsorbing oil vapour.*

in electronic tubes: the metal combines chemically with some gases and adsorbs most of them when condensing. The application of this technique to ultra-high vacuum is complicated by the facts that the resulting compounds may have too high a dissociation pressure and that the metals often contain too much gas, which is released during heating. Further, inert gases are not appreciably adsorbed at room temperature. However, it has been demonstrated repeatedly that pumping by evaporated-metal films is possible in ultra-high vacuum if it is preceded by thorough degassing at a high temperature.

Another pumping principle is that of ion pumping, which has already been referred to. In this, gas molecules are ionized, accelerated by an electric field, and buried in an ion collector. Some metal is sputtered off the collector by the ion bombardment; this metal adsorbs gas and so enhances the pumping effect. Such pumps have become increasingly popular for high-vacuum work, but in the ultra-high-vacuum region the pumping speed is greatly reduced.

A special application of ion bombardment is for the cleaning of surfaces by a gas discharge, a procedure used successfully in some thermonuclear machines. The ion bombardment of a surface releases gas molecules adsorbed at the surface and replaces them with the molecules of the bombarding gas. If this gas is an inert gas, such as helium, it is then possible to remove it by prolonged pumping.

Just as pumping by evaporated-metal coatings is an extension of the adsorption trap, so cryogenic pumping—that is, condensing of gases at a cold surface—is an extension of the cold trap. We have found in our laboratory that the pressure in a degassed ion-gauge that is sealed off from an unbaked system drops from the 10^{-7} to the 10^{-10} torr range if a portion of the wall is cooled with liquid nitrogen. The water vapour is rapidly 'pumped' by the cold spot, and the pumping speed of the gauge is large enough (10^{-3}–10^{-2} litres/sec) to cope with the evolution of helium and carbon monoxide. Cryogenic pumping is usually done with either liquid helium (boiling point −269°C) or liquid hydrogen (boiling point −253° C). P. A. Redhead [9] obtained pressures in the 10^{-13} torr range by dipping a finger-like extension of the vacuum system into liquid helium. At −269° C hydrogen has a vapour pressure of 10^{-6} torr: apart from helium, it is the only substance that still has an appreciable vapour pressure at that temperature. At −253° C only hydrogen, helium, and neon still have appreciable vapour pressures; the vapour pressure of carbon monoxide at that temperature is less than 10^{-10} torr. D. J. Santeler [10] has analysed this type of pumping and recommends it for large chambers for simulating conditions in space. He suggests the use of panels cooled with liquid hydrogen, these being shielded against the radiation from the chamber by other panels cooled with liquid nitrogen. Russian workers [11] have investigated two liquid hydrogen pumps having pumping speeds of 4000 and 13 000 litres/sec respectively. An advantage of cryogenic pumping is that high pumping speeds can be obtained by dispensing with the connecting pipe between the pump and the vacuum vessel and cooling part of the vessel wall.

REFERENCES

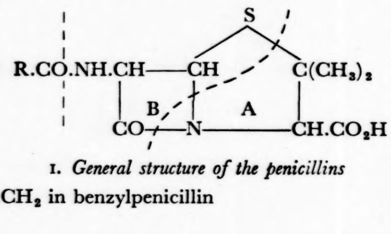
[1] ANDRADE, E. N. DA C. *Endeavour*, **16**, 29, 1957.
[2] Review article by D. ALPERT in 'Handbuch der Physik', Vol. XII, p. 609, 1958.
[3] GOTTLIEB, M. *Endeavour*, **19**, 62, 1960.
[4] HOBSON, J. P. and REDHEAD, P. A. *Can. J. Phys.*, **36**, 271, 1958.
[5] STEINHERZ, H. A. 'Ultra High Vacuum Techniques for Industrial Size Systems'. Transaction New York University Vacuum Metallurgy Symposium. (In press.) 1960.
[6] DREYER, K. and MARK, J. T. 'Ultra High Vacuum System Developments for the Model C-Stellarator'. *Vacuum Symp. Trans.*, 176, 1959.
[7] GROVE, D. J. *Vacuum Symp. Trans.*, 9, 1958.
[8] MUNDAY, G. L. *Nuclear Instr. Methods*, **4**, 367, 1959.
[9] REDHEAD, P. A. *Vacuum Symp. Trans.*, 148, 1958.
[10] SANTELER, D. J. 'Pressure Simulation of Outer Space'. Rep. No. 59GL228, September 1959. General Electric Co., Schenectady, New York.
[11] LAZAREV, B. G., BOROVIK, E. S., EYEDOROVA, M. F., and TSIN, N. M. Украïн. Жур. Физ., **2**, 175, 1957.

New penicillins, cephalosporin C, and penicillinase

E. P. ABRAHAM and G. G. F. NEWTON

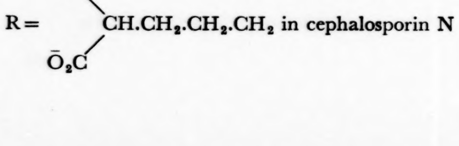
A serious limitation to the value of penicillin in medicine arose from the emergence of strains of *Staphylococcus aureus* that showed resistance to the common penicillins because they produced the enzyme penicillinase. In some hospitals the proportion of resistant strains has been as high as 80 per cent. Later, the staphylococcus was found to acquire resistance to other antibiotics. This article describes advances in the chemistry and biochemistry of penicillin and related compounds that suggest important new medical possibilities.

When the extensive Anglo-American work on the penicillins came to an end in 1946, the problem of producing benzylpenicillin from *Penicillium chrysogenum* on a commercial scale had been largely solved and the pharmaceutical industry was beginning to look for new antibiotics. The general structure of the penicillins (I) had been elucidated [1]. It was found to consist of a nucleus, containing a thiazolidine ring (A) fused to a β-lactam ring (B), to which was attached a side-chain (R.CO.) that varied from one penicillin to another. The structure could be dissected, as shown by the broken lines in I, into units of L-cysteine, valine, and a carboxylic acid, $R.CO_2H$; Sir Robert Robinson has remarked that the structural relationships of penicillin were always sun clear [2]. Experimental evidence for the biogenesis of the molecule from these units was, however, available only in the case of the side-chain. The addition of a variety of mono-substituted acetic acids to the culture fluid of *Penicillium chrysogenum* had been found to increase the yield of antibiotic and to result in the formation of a penicillin whose side-chain was derived from the acid added. For example, the addition of phenylacetic acid ($C_6H_5.CH_2.CO_2H$) greatly increased the yield of benzylpenicillin (I, $R = C_6H_5.CH_2$).

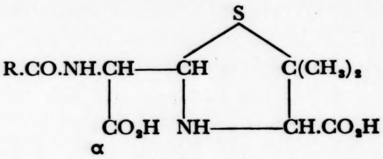


I. *General structure of the penicillins*

$R = C_6H_5.CH_2$ in benzylpenicillin



$$R = \begin{matrix} H_3\overset{+}{N} \\ \quad\diagdown \\ \quad\quad CH.CH_2.CH_2.CH_2 \\ \quad\diagup \\ \overset{-}{O}_2C \end{matrix}$$ in cephalosporin N



II. *Penicilloic acid formed from penicillin in the presence of penicillinase*

After several years in which little that was new and significant appeared, further advances began to be made. The initial reasons for these advances were largely academic in character, but, at a later stage, activity was undoubtedly stimulated by clinical problems that emerged during the extensive use of penicillin in medicine.

In clinical practice a serious problem arose from a change in the strains of *Staphylococcus aureus* that were causing staphylococcal infections. When penicillin was first introduced into medicine, most strains of staphylococci were highly sensitive to it. As time passed, resistant strains began to be isolated from patients, particularly in hospitals. The proportion of staphylococcal infections caused by resistant strains rose to more than 80 per cent in some hospitals, and the clinical problem was greatly increased by the fact that the staphylococcus showed a tendency to become highly resistant to other antibiotics as well. The staphylococci that were resistant to penicillin were found to produce the enzyme penicillinase, which destroys penicillin. Strains that produced penicillinase became more numerous by selection. They survived and multiplied when the sensitive strains were killed off, and in hospitals they were carried by the medical and nursing staff and passed from patient to patient. Staphylococci that do not produce penicillinase, but are resistant to penicillin for other reasons, can readily be obtained in the laboratory by the repeated subculture of sensitive organisms in the presence of increasing amounts of the drug. But strains of this type have not hitherto presented a clinical problem, perhaps because they are less virulent than their parents. It thus seemed reasonable to hope that a valuable new chemotherapeutic agent would be obtained if the penicillin molecule could be modified in such a way that it retained its high activity but lost its sensitivity to penicillinase.

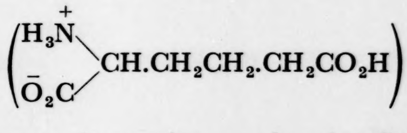
Penicillinase—discovered, in 1940, in the cells of *Escherichia coli* [3]—catalyses the hydrolytic opening of the β-lactam ring of a penicillin with the formation of an inactive penicilloic acid (II). Relatively large amounts of extracellular penicillinase were shown later to be formed by strains of *Bacillus cereus* and it is the enzyme from this source that was first subjected to detailed study. The synthesis of penicillinase by some strains of *B. cereus* proved to be inducible, occurring at a greatly increased rate when the cells came into contact with penicillin [4]. Until recently, staphylococcal penicillinase had received comparatively little attention, but it is now known that the enzyme produced by many strains of *Staphylococcus aureus* is partly extracellular and partly intracellular and that it is inducible. Penicillinases from different sources produce the same qualitative change in the penicillin molecule, but they may differ quantitatively in their enzymic activity.

The hydrolysis of a penicillin by penicillinase may be described in the normal terms of velocity constants for formation and dissociation of an enzyme-substrate complex and for the decomposition of the complex into enzyme and product. With a given concentration of enzyme, the rate of formation of product increases with the concentration of substrate until it eventually approaches a maximum, V_{max}, when virtually all the reactive sites on the enzyme molecules are occupied by substrate. The concentration of substrate required for the rate of reaction to reach half V_{max} is given by the Michaelis constant, K_m, which depends, at least in part, on the affinity of the substrate for the enzyme. With benzylpenicillin as the substrate, V_{max} is high and K_m is low, so that the penicillin is inactivated rapidly even in low concentrations. A useful decrease in sensitivity to penicillinase might thus result from a modification of the penicillin molecule which greatly increased K_m, or from one which greatly reduced V_{max}. In the former case, the new molecule might show too small an affinity for the enzyme to be hydrolysed at a significant rate in low concentrations, although in these concentrations it could nevertheless exert a bactericidal effect. In the case in which V_{max} was reduced, the affinity for the enzyme might still be high. If this were so, the new molecule should not only be resistant to inactivation by penicillinase but should compete for penicillinase with benzylpenicillin and so inhibit the inactivation of the latter.

In this article we shall discuss some of the post-war work in the penicillin field that has now impinged on the problem of the penicillinase-producing staphylococcus. One line of work has made it possible to obtain new penicillins by synthetic or semi-synthetic methods. Another, which developed from a study of antibacterial compounds produced by a species of *Cephalosporium*, has revealed the existence of a substance, cephalosporin C, which is structurally related to the penicillins but does not contain the classical penicillin nucleus.

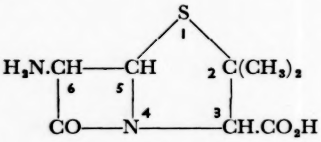
NEW PENICILLINS

By 1950, several hundred different penicillins had been obtained by adding various side-chain precursors, many of them derivatives of phenylacetic acid, to fermentations with *Penicillium chrysogenum*. In general, these substances did not differ greatly from benzylpenicillin in their range of antibacterial activity and they showed no significant resistance to penicillinase.

There was, however, a different antibiotic, produced by a species of *Cephalosporium*, that was later shown to be a penicillin with a new type of side-chain. G. Brotzu had isolated this fungus from the sea near a sewage outfall off the Sardinian coast and reported, in 1948, that a crude extract of its culture fluid exerted a beneficial effect on certain bacterial infections in man. His work was published in a local and little-known journal [5], but it was brought to the attention of Sir Howard Florey by a former British Public Health Officer in Sardinia. Brotzu sent a culture of his organism to Oxford, and further work was done on it both there and at the Antibiotics Research Station at Clevedon. The antibiotic that had first been encountered in Sardinia turned out to be a penicillin with a side-chain derived from D-α-aminoadipic acid  $\left(\begin{array}{l} H_3\overset{+}{N} \\ \bar{O}_2C \end{array}\!\!> CH.CH_2CH_2.CH_2CO_2H\right)$ [6]. This substance, (D-4-amino-4-carboxybutyl) penicillin, was named cephalosporin N; it is known in the United States as synnematin B [7].

The polar groups in the side-chain of cephalosporin N endow the molecule with extremely hydrophilic properties and a range of antibacterial activity that is quite different from that of the common penicillins. The new compound had less than one per cent of the activity of benzylpenicillin against the Oxford strain of *Staphylococcus aureus*, but was considerably more active against certain strains of *Salmonella typhi*. When the positive charge on the side-chain was removed by acylation of the amino group, the activity against the staphylococcus increased and that against the salmonella went down. It thus became clear that radical changes in biological properties could be brought about by certain types of change in the side-chain of the penicillin molecule. However, cephalosporin N was rapidly destroyed by penicillinase from *Staphylococcus aureus* and *Bacillus cereus*, V_{max} being about half that for benzylpenicillin and K_m with the latter enzyme being near that for benzylpenicillin [8]. Cephalosporin N thus showed no promise of being useful for the treatment of infections caused by penicillin-resistant staphylococci.

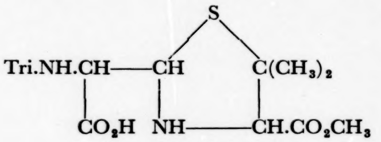
So far, the types of penicillin obtainable had been restricted by the specificity of enzymes involved in the incorporation of the side-chain into the molecule. In general, only monosubstituted acetic acids with non-polar groupings ($R.CH_2.CO_2H$) appeared to be utilized as side-chain precursors by *Penicillium chrysogenum*, while only one penicillin, with a side-chain derived from D-α-aminoadipic acid, appeared to be synthesized by the *Cephalosporium* species. The production of new penicillins by a method to which these apparent restrictions did not apply was made possible by the isolation of the nucleus of the penicillin molecule, 6-aminopenicillanic acid (iii).



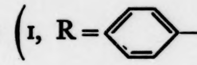
iii. 6-*Aminopenicillanic acid* (*the penicillin nucleus*)

The suggestion that the penicillin nucleus was produced in fermentations with *Penicillium chrysogenum* to which no side-chain precursor had been added was first made by K. Kato, in Japan, who found that a substance was present in these fermentations which showed some of the chemical reactions of benzylpenicillin but not its antibacterial activity [9]. In 1950, K. Sakaguchi and S. Murao, also in Japan, had stated that the penicillin nucleus could be obtained by the action of an amidase on benzylpenicillin [10]. The amidase was reported to be formed by a strain of *Penicillium chrysogenum* and to catalyse the hydrolysis of the phenylacetyl side-chain. But difficulty was experienced in repeating these experiments in other laboratories and for some time their validity was doubted.

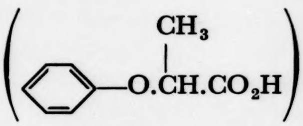
The first independent announcement of the existence of 6-aminopenicillanic acid came from J. C. Sheehan, who reported that the compound had been obtained by total synthesis [11]; this represented the culmination of an extensive series of investigations. Penicilloic acids had been synthesized during the war-time work on penicillin, but attempts to reconstruct the β-lactam ring from them had been frustrated by the ease with which an alternative ring closure, to yield an oxazolone, occurred between the α-carboxyl group and the amide group of the side-chain. The solution of the problem was found in use of side-chains from which oxazolone formation did not occur, together with a new ring-closing agent, dicyclohexylcarbodiimide. 6-Aminopenicillanic acid was finally obtained from a trityl (triphenylmethyl) derivative (iv). After formation of the β-lactam ring, the ester and trityl groups were hydrolysed by alkali and acid respectively under mild conditions [12].



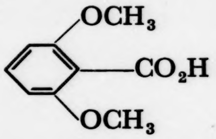
iv

This total synthesis of 6-aminopenicillanic acid made a gratifying addition to the achievements of synthetic organic chemistry, but it involved a number of stages in which the yields were not high and it did not provide a convenient route to the production of large quantities of the compound. 6-Aminopenicillanic acid was first made readily available by the discovery by F. R. Batchelor, F. P. Doyle, J. H. C. Nayler, and G. N. Rolinson, that it could be isolated in crystalline form from fermentations of *Penicillium chrysogenum* to which no side-chain precursor had been added [13]. More recently, amidases that catalyse the hydrolytic removal of the side-chain from penicillins, with the formation of 6-aminopenicillanic acid, have been shown to exist in a variety of micro-organisms [14]. At least two types of enzyme have been detected. One, which occurs in certain fungi and actinomycetes, hydrolyses phenoxymethyl penicillin  $(\mathrm{I},\ R = C_6H_5\text{—}O.CH_2)$, *n*-amylpenicillin ($\mathrm{I}$, $R = C_5H_{11}$), and *n*-heptylpenicillin (I, $R = C_7H_{15}$) more rapidly than it does benzylpenicillin. Another, which occurs in bacteria of the genera *Escherichia* and *Alcaligenes*, hydrolyses benzylpenicillin more rapidly than phenoxymethylpenicillin.

6-Aminopenicillanic acid has a very low antibacterial activity, but it can be readily acylated, for example by treatment with acid chlorides, to yield penicillins, and several thousand new penicillins have already been obtained in this way. As a result of the work of Batchelor and his colleagues, two of these substances have now been introduced into medicine. The first, α-phenoxyethylpenicillin, has a side-chain derived from α-phenoxypropionic acid



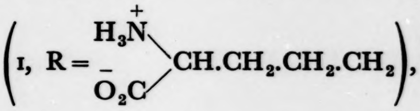
Its maximum rate of hydrolysis by penicillinase is not greatly less than that of benzylpenicillin, but it is useful (like phenoxymethylpenicillin) for oral administration, because it is relatively well absorbed from the gastro-intestinal tract. The second, 2:6-dimethoxyphenylpenicillin, with a side-chain derived from 2:6-dimethoxybenzoic acid



has more interesting properties [15]. It has only about one-fiftieth of the activity of benzylpenicillin against penicillin-sensitive strains of *Staphylococcus aureus*, but its maximum rate of hydrolysis by penicillinase from *Bacillus cereus* is about thirty times less than that of benzylpenicillin and by staphylococcal penicillinase is less still. It induces the formation of penicillinase by both organisms. It is a strong competitive inhibitor of the inactivation of benzylpenicillin by penicillinase from *Bacillus cereus*, but not by penicillinase from *Staphylococcus aureus*, and thus appears to have a much lower affinity for the staphylococcal enzyme. This may diminish its rate of hydrolysis yet further, relative to that of benzylpenicillin, when it comes into contact with penicillinase-producing staphylococci in concentrations no greater than those required to exert an antibiotic effect.

CEPHALOSPORIN C

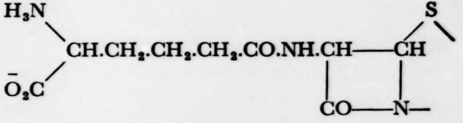
During work on the structure of cephalosporin N



$$\left(\mathrm{I},\ R = \begin{matrix} H_3\overset{+}{N} \\ {}^{-}O_2C \end{matrix} > CH.CH_2.CH_2.CH_2\right)$$

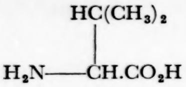
, we found that partially purified samples of this penicillin were contaminated by a compound that showed an absorption maximum at 2600 Å in ultra-violet light. The new compound, which was named cephalosporin C, was isolated as a crystalline sodium salt. It proved to have antibacterial activity, but to be only about one-tenth as active as cephalosporin N against most organisms. Cephalosporin C was subsequently shown to be present in culture fluids of the *Cephalosporium* sp. that produced cephalosporin N. At the time of its discovery, its concentration in these culture fluids was too low for it to be detected by measurements of antibacterial activity.

Cephalosporin C was found to contain two more carbon atoms and two more oxygen atoms in its molecule than does cephalosporin N. Like cephalosporin N, it contained a residue of α-aminoadipic acid linked to the rest of the molecule through its δ-carboxyl group and it showed an infra-red spectrum with a strong band at 5·62 μ, characteristic in the penicillin family of the stretching vibration of the C═O group in the fused β-lactam ring. These properties, together with those of a variety of degradation products, indicated that cephalosporin C could be assigned the partial structure V.



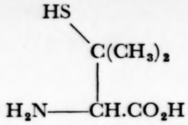
V

This partial structure left seven carbon atoms to be accounted for. Two of these carbon atoms were present as an acetoxyl group ($CH_3CO.O$). The remaining five atoms, like five of the carbons in the thiazolidine ring of the penicillins (I, ring A), formed part of a fragment that yielded valine (VI) on vigorous hydrogenolysis with Raney nickel. Nevertheless, this fragment of cephalosporin C differed from the corresponding fragment of the



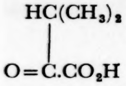
$H_2N-\overset{HC(CH_3)_2}{CH}.CO_2H$

VI. *Valine*



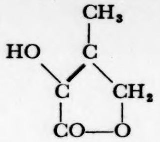
$H_2N-\overset{HS-C(CH_3)_2}{CH}.CO_2H$

VII. *Penicillamine*

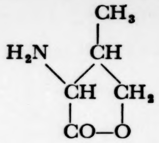


$O=\overset{HC(CH_3)_2}{C}.CO_2H$

VIII. α-*Ketoisovaleric acid*



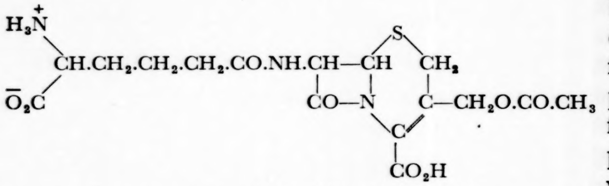
X. α-*Hydroxy*-β-*methylbutenolide*



XI. γ-*Hydroxyvaline lactone*

penicillins, for the penicillins yielded the characteristic amino acid penicillamine (D-β-thiolvaline, VII) on hydrolysis while cephalosporin C did not. During hydrolysis of cephalosporin C, a nitrogen atom was eliminated from the five-carbon fragment in the form of ammonia and when hydrolysis was preceded by mild hydrogenolysis, α-ketoisovaleric acid (VIII) was one of the products. Furthermore, a nuclear magnetic resonance spectrum, determined by P. Higham and R. E. Richards, showed that cephalosporin C, unlike the penicillins, contained no gem-dimethyl group ($C(CH_3)_2$), despite the presence of such a group in two of its degradation products (VI and VIII).

At this stage we proposed for cephalosporin C, as a working hypothesis, the structure IX, in which sulphur was linked to a γ-carbon of the valine-yielding fragment to form a six-membered ring [16]. The proposed structure accounted for the behaviour of the molecule on hydrolysis and hydrogenolysis and for its infra-red and nuclear magnetic resonance spectra. It could not have been predicted that such a structure would show an absorption maximum at as long a wavelength as 2600 Å in ultra-violet light, but no adequate model-compounds were available with which a comparison could be made.



IX. *Structure proposed for cephalosporin C*

Soon after this point had been reached, D. Hodgkin and E. N. Maslen, who had been carrying out an X-ray crystallographic analysis of the sodium salt of cephalosporin C, were able to discern the presence of a six-membered sulphur-containing ring in the molecule [17]. From then on new degradation products were rapidly isolated that gave further support to structure IX. Among them were α-hydroxy-β-methylbutenolide (X) and γ-hydroxyvaline lactone (XI), both of which were obtained after mild hydrogenolysis and subsequent treatment with hot acid.

At the same time, the X-ray crystallographic analysis made rapid progress and soon reached a stage at which the relative positions of the atoms in the molecule were defined, although not with great accuracy. These positions, as shown by the contours representing electron-density levels in figure 1, corresponded with structure IX.

Cephalosporin C thus contains a fused β-lactam-dihydrothiazine ring system in place of the β-lactam-thiazolidine ring system of the penicillins. The positions of the atoms in the β-lactam ring, and of those directly attached to this ring, are very similar in the two structures, although the structures differ considerably in other respects. The dihydrothiazine ring does not appear to have been previously encountered in nature.

Although the chemical properties of cephalosporin C appeared to be sufficiently interesting in themselves to be worth detailed exploration, interest in this antibiotic was enhanced by its biological properties. Its sodium salt was even less toxic to mice than is sodium benzylpenicillin. Its mode of action was similar to that of the penicillins: like the latter, it brought about the lysis of growing staphylococci and appeared to inhibit the synthesis of staphylococcal cell walls [23]. It was a powerful inducer of the formation of penicillinase by *Staphylococcus aureus* and *Bacillus cereus*. In contrast, its maximum rate of hydrolysis was about five thousand times smaller than that of benzylpenicillin in the presence of purified penicillinase from *Bacillus cereus* and also much smaller in the presence of staphylococcal penicillinase. There was no indication that the striking reduction in V_{max} was accompanied by any comparable decrease in affinity for penicillinase from *Bacillus cereus*, since cephalosporin C competitively inhibited the hydrolysis of benzylpenicillin by this enzyme [8]. However, the hydrolysis of benzylpenicillin by extracellular penicillinase from a strain of *Staphylococcus aureus* was not significantly inhibited by cephalosporin C in concentrations similar to those of the penicillin.

Cephalosporin C was as active *in vitro* against penicillinase-producing strains of *Staphylococcus aureus* as against the Oxford strain that did not produce penicillinase. Nevertheless, its activity

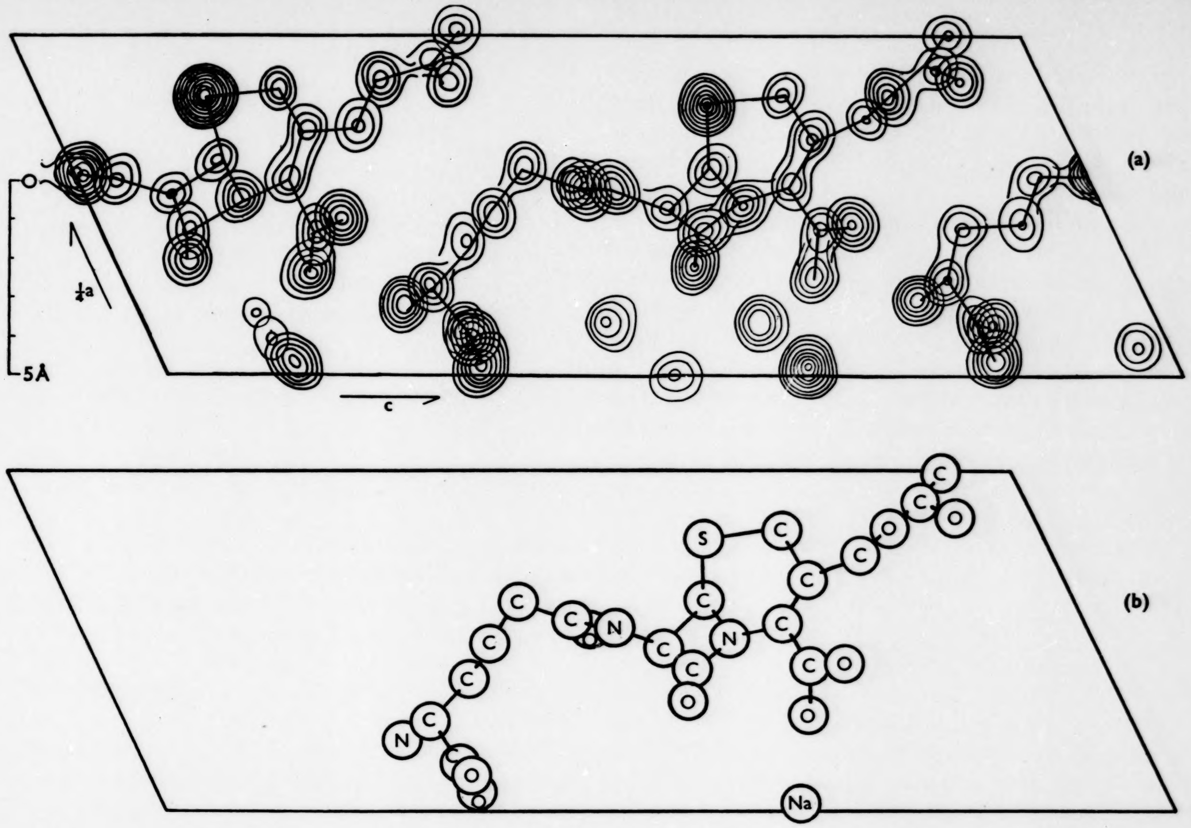
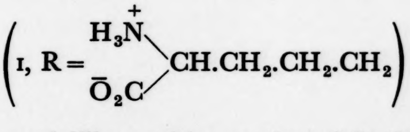


FIGURE 1 – (a) *The structure of the sodium salt of cephalosporin C as it appears from the three-dimensional Fourier synthesis seen down the b axis. The figure represents the asymmetric unit (two molecules in the crystal). The contours represent electron density levels corresponding to the various atoms. Atoms that are not labelled represent the oxygen atoms of water molecules.* (b) *The structure of the sodium salt of cephalosporin C drawn from the atomic positions in* 1a. (*From the results of an X-ray crystallographic analysis by D. Hodgkin and E. N. Maslen.*)

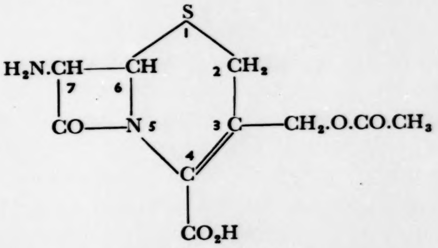
against the Oxford strain was very low, being only about 0·1 per cent of that shown by benzylpenicillin. It thus seemed worth while to explore the possibility of obtaining a derivative of cephalosporin C that retained the resistance of the parent compound to penicillinase but showed a much higher activity against the staphylococcus.

Since benzylpenicillin was more than one hundred times as active as cephalosporin N



$$\left(\text{I, R} = \begin{matrix} \overset{+}{H_3N} \\ \bar{O}_2C \end{matrix} \rangle CH.CH_2.CH_2.CH_2\right)$$

against penicillin-sensitive strains of *Staphylococcus aureus*, we thought it likely that a very large increase in activity would result from an exchange of the α-aminoadipyl side-chain in cephalosporin C for a side-chain derived from phenylacetic acid or one of its derivatives. An attempt was made to remove the side-chain from cephalosporin C, by mild acid hydrolysis, without changing the remainder of the molecule. This attempt was successful and although the process was a highly inefficient one, it enabled the nucleus of cephalosporin C, 7-aminocephalosporanic acid (XII), to be isolated in relatively pure form [18].



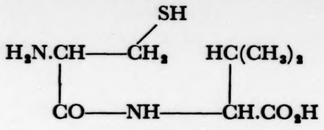
XII. 7-*Aminocephalosporanic acid* (*the cephalosporin C nucleus*)

When 7-aminocephalosporanic acid was acylated with phenylacetyl chloride it yielded a phenylacetyl derivative which was several hundred times as active as cephalosporin C, and had about one-fifth of the activity of benzylpenicillin against the Oxford staphylococcus. However, unlike benzylpenicillin, this derivative was highly active *in vitro* against strains of staphylococci that produced penicillinase.

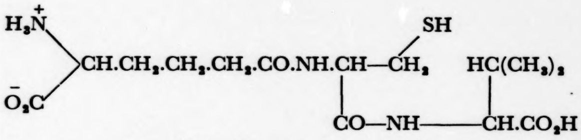
The similarity in the effect produced by these changes of side-chain on antibacterial activity in the cephalosporin C and penicillin families was found to extend further. Penicillins formed by coupling phenoxyacetic acid or α-phenoxypropionic acid to 6-aminopenicillanic acid are at least as active as benzylpenicillin against the staphylococcus *in vitro*, whereas those formed by coupling dimethoxybenzoic acid, propionic acid, or acetic acid are considerably less active. Comparable differences in activity were found among the corresponding derivatives of 7-aminocephalosporanic acid.

BIOGENETIC RELATIONSHIPS

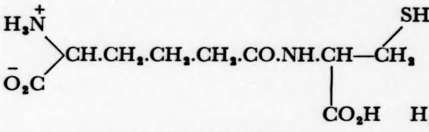
The structural relationships of benzylpenicillin, cephalosporin N, 6-aminopenicillanic acid, and cephalosporin C indicate that these antibiotics are related biogenetically. However, benzylpenicillin and 6-aminopenicillanic acid are produced by *Penicillium chrysogenum*, whereas the two cephalosporins, with side-chains derived from D-α-aminoadipic acid, are produced by a species of *Cephalosporium*. The differences in enzymic constitution that are responsible for the fact that the two types of fungi produce different, though structurally related, compounds have still be to determined, but there is an indication that α-aminoadipic acid plays a role in the biosynthesis of penicillins produced by *Penicillium chrysogenum* as well as in that of cephalosporin N and cephalosporin C. H. R. V. Arnstein and D. Morris showed that L-cysteinyl-L-valine (XIII) could be used by *Penicillium chrysogenum* for the biosynthesis of benzylpenicillin without prior hydrolysis into cysteine and valine. The dipeptide was not used as effectively, however, as would have been expected had it been an intermediate on the only biosynthetic pathway leading to the antibiotic. A possible explanation of this finding seemed to be provided by the discovery of L-α-aminoadipic acid and δ-(α-aminoadipyl)cysteinylvaline (XIV) in the mycelium of *Penicillium chrysogenum* [19]. The tripeptide (XIV) has a formal resemblance to glutathione (γ-glutamylcysteinylglycine) and might be synthesized in similar stages from its component amino acids. In that case, a major biosynthetic pathway would involve the condensation of α-aminoadipic acid with L-cysteine to form δ-(α-aminoadipyl) cysteine (XV) and the subsequent condensation of the latter with L-valine (XVI). But an alternative pathway would involve the transfer of α-aminoadipic acid from δ-(α-aminoadipyl) peptides to L-cysteinyl-L-valine (XIII).



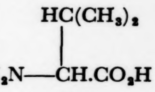
XIII. *Cysteinylvaline*



XIV. δ-(α-*Aminoadipyl*)*cysteinylvaline*



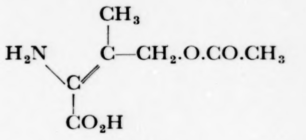
XV. δ-(α-*Aminoadipyl*)*cysteine*



XVI. *Valine*

It has been suggested that the cysteinylvaline fragment of the tripeptide (XIV) undergoes oxidative intramolecular condensations to form the penicillin ring system and that enzymes occur in *Penicillium chrysogenum*, but not in *Cephalosporium*, which can bring about, at some stage in the process, the hydrolytic removal of the α-aminoadipic acid residue or its replacement by a residue of phenylacetic acid [19]. If this were so, L-cysteinyl-L-valine (XIII) would not be an obligatory intermediate in the biosynthesis of penicillins and the extent of its incorporation into the penicillin molecule might depend on the extent to which it was converted to δ-(α-aminoadipyl)cysteinylvaline (XIV) by transpeptidation. Moreover, the fact that the α-aminoadipic acid in cephalosporin N has the D-configuration, while α-aminoadipic acid in the mycelium of *Penicillium chrysogenum* is preponderantly L, might have some bearing on the apparent failure of the *Cephalosporium* to produce benzylpenicillin or 6-aminopenicillanic acid. In a number of micro-organisms, enzymes have been found that catalyse the condensation of 6-aminopenicillanic acid (III) with phenylacetic and related acids, as well as the hydrolytic removal of the latter from the corresponding penicillins [14]. It is thus possible that acylation of 6-aminopenicillanic acid represents a final stage in the biosynthesis of benzylpenicillin by *Penicillium chrysogenum.*

The structure of cephalosporin C can be dissected into D-α-aminoadipic acid, L-cysteine, and the O-acetyl derivative of a hypothetical αβ-dehydro-γ-hydroxyvaline (XVII). This third structural unit might be formed by the oxidation of a valine residue in an intermediate that is also involved in the biosynthesis of cephalosporin N.



XVII

These hypotheses, however, remain to be tested. What has been established is the nature of a number of new compounds which form part of the biochemistry of penicillin or may plausibly be assumed to do so. Among them are δ-(α-aminoadipyl)cysteinylvaline (XIV), cephalosporin N, 6-aminopenicillanic acid, and cephalosporin C. But the discovery of such compounds does not carry with it a knowledge of the way in which they are linked in biosynthetic processes.

THE PROBLEM OF THE PENICILLINASE-PRODUCING STAPHYLOCOCCUS

We now know that specific changes in either the side-chain or the thiazolidine ring of the natural penicillins may yield compounds that have antibacterial activity but a greatly decreased sensitivity to penicillinase. 2:6-Dimethoxyphenylpenicillin, in which the penicillin nucleus, 6-aminopenicillanic acid (III), is condensed with 2:6-dimethoxybenzoic acid, has already proved to be clinically useful for the treatment of infections by penicillinase-producing staphylococci [20]. Cephalosporin C (IX), in which the nucleus is 7-aminocephalosporanic acid, has been shown to protect mice from such infections [21], and compounds in which 7-aminocephalosporanic acid is condensed with phenylacetic, α-phenoxypropionic, or similar acids promise to be effective in smaller doses than cephalosporin C itself.

While these are significant advances, it should not be assumed that they have given us ideal chemotherapeutic agents or that they will necessarily provide a permanent solution to the clinical problems now presented by the staphylococcus. 2:6-Dimethoxyphenylpenicillin is intrinsically much less active than benzylpenicillin against *Staphylococcus aureus* and must be injected into a vein or muscle in relatively large amounts. The resistance of the new compounds to penicillinase, though high, is not absolute and it may vary with the source of the enzyme. Penicillinase produced by *Staphylococcus aureus* is not identical with that produced by *Bacillus cereus*, and the latter organism, at least, is itself able to produce enzymes that differ quantitatively in their effect on cephalosporin C. Moreover, the amide linkage of side-chain to nucleus, as well as the β-lactam ring, may be susceptible to enzymic hydrolysis. One possible consequence of bringing the new compounds into general clinical use would be the emergence of staphylococci that produced new penicillinases, or other enzymes, by which these compounds would be readily inactivated. Another would be the appearance, in patients, of staphylococci that had acquired a different type of resistance. The fact that it is the penicillinase-producing organism that has so far impaired the value of penicillin for the treatment of staphylococcal infections provides no guarantee that non-penicillinase producers will not acquire clinically significant resistance to penicillins with new side-chains. The staphylococcus becomes resistant to many other antibiotics *in vivo* without apparently producing enzymes by which these antibiotics are destroyed.

However, the discovery that quite extensive changes may be made in the penicillin molecule without virtual destruction of its antibacterial activity raises the hope that further progress in this field is possible, and that attempts to learn more of the relationship between structure and biological properties among derivatives of 6-aminopenicillanic acid, 7-aminocephalosporanic acid, and related substances will prove rewarding. Antibacterial activity and resistance to penicillinase are not the only properties that are of interest in this connection. For example, a substance with a low activity, but a high resistance to staphylococcal penicillinase, might have additional value if it combined strongly enough with the penicillinase to be a powerful inhibitor of the action of the enzyme on the normal penicillins *in vivo*. A substance with a limited resistance to penicillinase would be more effective if it failed to induce the formation of the enzyme by *Staphylococcus aureus* than if, like benzylpenicillin, it acted as an inducer. The finding of substances with such properties remains a possibility. Both cephalosporin C and 2:6-dimethoxyphenylpenicillin are competitive inhibitors of the inactivation of benzylpenicillin by penicillinase from *Bacillus cereus*. The structural requirements of effective competitive inhibitors appear to be more rigid with staphylococcal penicillinase than with penicillinase from *Bacillus* *cereus*, but the phenylacetyl derivative of 7-aminocephalosporanic acid has some inhibitory action on the hydrolysis of benzylpenicillin by the extracellular staphylococcal enzyme [22]. 2:6-Dimethoxyphenylpenicillin, cephalosporin C, and a number of other derivatives of 7-aminocephalosporanic acid are inducers of penicillinase, but some of them are more efficient inducers than others in relation to their antibacterial activity.

Within the last few years we have learned much about the effects of certain modifications of the penicillin structure on antibacterial activity, maximum rate of hydrolysis by penicillinase, ability to combine with penicillinase, and ability to induce the formation of penicillinase. It appears that a change in one of these properties is not necessarily accompanied by a parallel change in others, and that the behaviour of a compound to penicillinase may vary with the source of the enzyme. We now require more extensive information about the variation of such properties with structure, and a knowledge, in addition, of the structural features of the site of enzymic activity in penicillinases. When these have been obtained, it may be possible to interpret some of the present findings in terms of molecular structure and to predict the structures of further compounds that would have useful biological properties.

REFERENCES

[1] 'The Chemistry of Penicillin' (edited by H. T. Clarke, J. R. Johnson, and Sir Robert Robinson). Princeton University Press, Princeton. 1949.

[2] Robinson, R. 'The Structural Relations of Natural Products'. Oxford University Press, London. 1955.

[3] Abraham, E. P. and Chain, E. *Nature, Lond.*, **146**, 837, 1940.

[4] Pollock, M. R. in 'The Enzymes', Vol. I, p. 619; Vol. IV, p. 269. Academic Press Inc., New York. 1959.

[5] Brotzu, G. *Lav. Ist. Igiene, Cagliari*, 1948.

[6] Abraham, E. P., Newton, G. G. F., Crawford, K., Burton, H. S., and Hale, C. W. *Nature, Lond.*, **171**, 343, 1953.
Newton, G. G. F. and Abraham, E. P. *Biochem. J.*, **58**, 103, 1954.

[7] Abraham, E. P., Newton, G. G. F., Olson, B. H., Schuurmans, D. M., Schenk, J. R., Hargie, M. P., Fisher, M. W., and Fusari, S. A. *Nature, Lond.*, **176**, 551, 1955.

[8] Abraham, E. P. and Newton, G. G. F. *Biochem. J.*, **63**, 628, 1956.

[9] Kato, K. *J. Antibiotics, Japan*, A.6, 130, 184, 1953.

[10] Sakaguchi, K. and Murao, S. *J. agric. chem. Soc. Japan*, **23**, 411, 1950.
Murao, S. and Karshi, H. N. *Ibid.*, **29**, 400, 1955.

[11] Sheehan, J. C. In 'Amino acids and peptides with antimetabolic activity'. Ciba Foundation Symposium (edited by G. E. W. Wolstenholme and C. M. O'Connor). P. 258. Churchill, London. 1958.

[12] Sheehan, J. C. and Henery-Logan, K. R. *J. Amer. chem. Soc.*, **81**, 5838, 1959.

[13] Batchelor, F. R., Doyle, F. P., Nayler, J. H. C., and Rolinson, G. N. *Nature, Lond.*, **183**, 257, 1959.

[14] Rolinson, G. N., Batchelor, F. R., Butterworth, D., Wood, J. C., Cole, M., Eustace, G. C., Hart, M. V., Richards, M., and Chain, E. B. *Ibid.*, **187**, 236, 1960.
Claridge, C. A., Gourevitch, A., and Lein, J. *Ibid.*, **187**, 237, 1960.
Huang, H. T., English, A. R., Seto, T. A., Shull, G. M., and Sobin, B. A. *J. Amer. chem. Soc.*, **82**, 3790, 1960.
Kaufmann, W. and Bauer, K. *Naturwissenschaften*, **20**, 474, 1960.

[15] Rolinson, G. N., Stevens, S., Batchelor, F. R., Wood, J. C., and Chain, E. B. *Lancet*, **ii**, 564, 1960.

[16] Abraham, E. P. and Newton, G. G. F. *Biochem. J.* (In press.) 1961.

[17] Hodgkin, D. and Maslen, E. N. *Ibid.* (In press.) 1961.

[18] Loder, P. B., Newton, G. G. F., and Abraham, E. P. *Ibid.* (In press.) 1961.

[19] Arnstein, H. R. V. and Morris, D. *Ibid.*, **76**, 357, 1960.

[20] Douthwaite, A. H. and Trafford, J. A. P. *Brit. Med. J.*, 687, 1960, and the six following papers.
Brown, D. M. and Acred, P. *Lancet*, **ii**, 568, 1960.

[21] Florey, H. W. *Giorn. Microbiol.*, **2**, 361, 1956.

[22] Crompton, B., Jago, M., Abraham, E. P., and Newton, G. G. F. (Unpublished experiments.)

[23] Abraham, E. P. *Endeavour*, **18**, 212, 1959.

Echolocation by bats

J. D. PYE

Spallanzani established, in 1794, that bats orientate themselves by use of the ears; he presumed that sounds were involved. It has now been shown that bats do in fact navigate, and find their food, by means of an echolocation system using sounds of very high frequency. The process is very rapid, accurate, and resistant to jamming; the author of this article suggests a probable mechanism based on a general theory applicable to nearly all Microchiroptera.

An important part of man's technological progress has involved the development of a variety of devices to aid and supplement his own sensory apparatus. Many of these have acquired great practical importance in helping him to find his way about, especially under difficult conditions. Thus telescopes, night-glasses, and infra-red 'sniperscopes' increase the definition, sensitivity, and spectral response of the eyes, while microphones and hydrophones aid the ears. All these examples are 'passive' in that they rely on signals from external sources, but some other instruments show no such dependence; they radiate energy themselves and create their own signals by interaction with the surroundings. This 'active' group includes radar, asdic (or sonar), searchlights, and even the humble hand-torch. Devices of this type are achieving increased prominence in many fields, and are often used with advantage to augment the passive ones.

It should hardly be surprising, therefore, to find that a similar development has occurred within the sensory apparatus of some animals. Specialization of the sense organs alone is insufficient to allow the full exploitation of certain habitats, and many examples are now known of active sensory systems that produce their own exploratory energy. Some deep-sea fishes may use their luminescent organs to see their way about. Other fish, such as the Gymnotids and Mormyrids, both fresh-water families, have developed electrical methods of orientation with remarkably high sensitivity [1, 2]; pulses of current are produced by the electric organs, and specialized receptors appear to detect impedance changes that distort the field around the body. The blind cave-fish (*Anoptichthys*) uses the lateral-line organs to detect reflections of pressure waves from its own swimming movements. This is really a semi-active mechanism, as the waves are not produced deliberately, but several of the higher vertebrates do produce sounds specially for their acoustic reflections. At least two genera of birds, the Venezuelan oil bird, *Steatornis* [3], and the 'birds-nest soup' swiftlet, *Collocalia* [4, 5], and also fruit bats of the genus *Rousettus* [6–9], can fly safely in complete darkness, guiding themselves by listening to the echoes of clicking noises made with the tongue. Some porpoises and dolphins make noises that are almost certainly used for underwater navigation, and the faculty may well be widespread among the Cetacea [10, 11]. But true echolocation at present appears to be most highly developed in bats of the sub-order Microchiroptera, most of which are insectivorous and nocturnal.

This was the first example of echolocation to be discovered. In 1794, L. Spallanzani established that bats depend for orientation almost completely on hearing and hardly at all on sight. A series of elegant, if cruel, experiments showed that bats with their ears plugged were helpless, but that blinded bats flew normally and were able to catch their prey when released [12]. H. Hartridge suggested in 1920 [13] that an echolocation mechanism using sounds of very high frequency might explain this paradox, and these sounds were detected by G. W. Pierce and D. R. Griffin in 1938 [14].

It has now been established beyond all doubt that this is the means by which bats navigate and find their food, and an account of the experiments performed by many workers over the last twenty years has been published by Griffin [15]. It has been shown that small bats can fly at speed through barriers of vertical wires only 0·4 mm in diameter and spaced only one wing-span apart. They achieve this with a collision rate far below that expected from random movement, and the collisions that do occur are often only slight ones, such as a light brush with the wing tip. When feeding, the bats can catch small insects, such as mosquitoes, at the rate of one every ten seconds for up to 30 minutes [16]. Each capture is accompanied by intensified emission of sound by the bat; only with very noisy prey does the bat appear to listen passively to the insect's wing-beats. The complete manœuvre may be completed in less than half a second, so that the information must be made available to the animal extremely quickly.

Photographs taken by American workers have shown that the bat pursues its prey with great accuracy at close range. If slight errors are made, so that the insect cannot be seized with the mouth, it may be scooped up by the tip of a wing [17]. All this can be accomplished in complete darkness or when the eyes are covered, but when the ears are carefully plugged even a seeing bat in a good light is completely disorientated and blunders into large objects.

The bat is therefore a highly successful self-directed missile that relies on its targets for fuel. But the analogy is not complete, for bats show an extraordinary resistance to jamming. Hunting often occurs with apparent success in highly compromising situations, such as in heavy rain, or among the foliage of trees and hedges. In certain parts of the world, enormous numbers of bats emerge together each night from their roosting places in caves: the background noise and multiplicity of echoes in these conditions would appear to be extremely confusing, yet accidents rarely happen and the interception of prey is continued.

Some species have been forced to fly in an artificial sound-field of 'white' noise whose frequency band covered that used by the bats. Wires of 0·5 mm diameter were avoided almost as well as in the quiet, and well above the expectation for random flight, even when the noise intensity was some 30 000 times greater than the calculated intensity of the echoes [15, 18]. This performance shows an extremely effective rejection of unwanted signals that at first sight appears difficult to explain. However, recent investigations into the 'cocktail party' effect, in which single conversations can be distinguished from a background of noise and chatter, suggest that two ears may be better than one. A model employing two microphones and a non-linear method of correlation has already shown considerable improvement over the theoretical limit for linear devices [19]. Development of this decision theory may help in an understanding of the bat's ability to deal with similar problems.

So far only two families of bats have been investigated very thoroughly, and they appear to have rather different methods of acoustic orientation. Details of the Vespertilionidae are known from the work of R. Galambos, D. R. Griffin, A. D. Grinnell, A. Novick, and others in North America, and of S. Dijkgraaf in Europe, while the European and African Rhinolophidae (horseshoe bats) have been the special study of F. P. Möhres and E. Kulzer. Only these two types can be described here, but other families that have been examined are often similar to these, or are to various extents intermediate in character [20–22].

The orientation sounds used by all Microchiroptera are produced vocally in the larynx. The specialized structure of this organ was described in detail by M. H. A. Robin [23] and by H. Elias [24], although the functional significance of their findings was not apparent at the time. Ossification and fusion of the cartilages has occurred, and the intrinsic muscles are very well developed, especially the cricothyroids, which apply tension to the two pairs of vibrating membranes. The membranes themselves are short and extremely light, permitting the production of very high frequencies. In the Rhinolophidae there are three resonating chambers opening from the trachea, and the glottis is ring-shaped and can be raised to fit into a cartilaginous ring around the internal nares. The Vespertilionidae, with few exceptions, produce their sounds through the mouth and over a fairly wide angle, while in the Rhinolophidae emission occurs through the nostrils and is beamed forwards. This is brought about by the nose-leaf structure, on the snout (figure 3), which acts as a horn and reflector, and by the nostrils, which are a half-wavelength apart, and cause interference laterally. The nose-leaf is capable of some movement, to direct the beam in different directions, and of changes of shape that may alter the pattern of emission to some extent [25].

Both families emit the sounds in discrete pulses that may attain high energy-levels. Recordings made 10 cm from a Vespertilionid show peaks reaching 60–170 dynes/cm^2, roughly equivalent to the level close to a pneumatic road-drill and rather more than an untrained person can produce by shouting. Rhinolophids may produce even higher intensities. The pulses of the Vespertilionidae are variable in duration, according to circumstances, but are generally very short. The typical pulse of a bat flying indoors lasts only 1–4 msec (figure 2, *a* and *b*); some species may produce longer pulses in the open air and shorten them when obstacles or prey are approached. The pulse-repetition rate is variable, and can change from less than 10 per second, when cruising, to

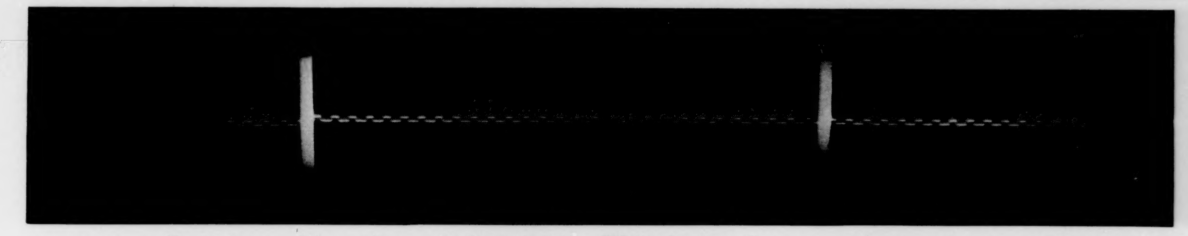
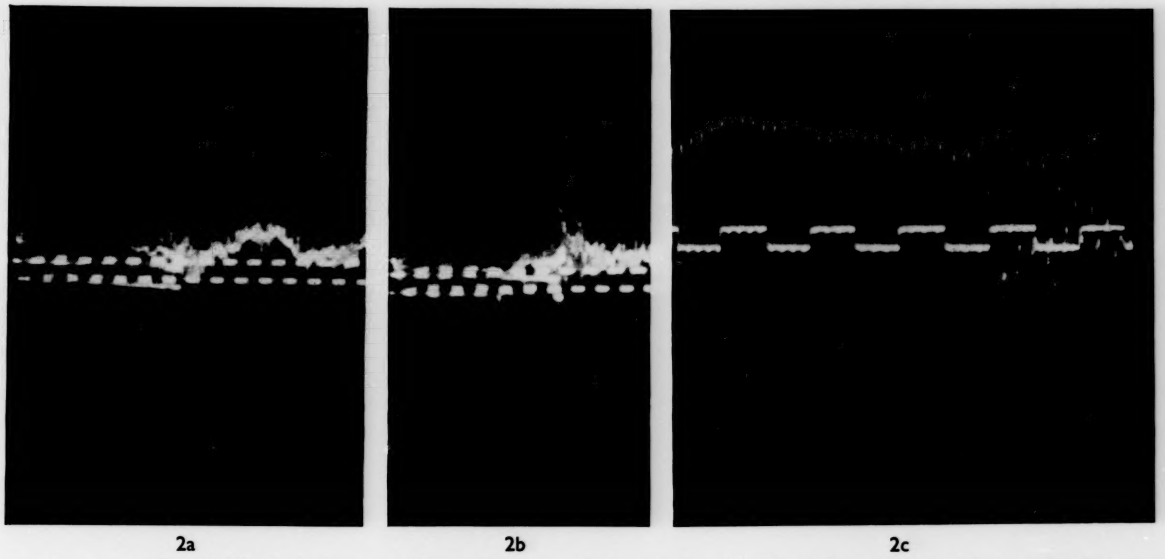


FIGURE 1 – *Part of an oscillograph record of a pulse from* Rhinolophus ferrum-equinum, *the greater horseshoe bat. The time bars are* 0·1 *msec long, and a total of* 54 *msec has been removed from the two gaps in the trace. Frequency is very constant but falls in the last* 1·5 *msec. The trace reads from left to right.*



2a 2b 2c

FIGURE 2 – (a) *and* (b) *Cathode-ray oscillograph traces of pulses produced by* Nyctalus noctula (*Vespertilionidae*) *indoors. The horizontal bars of the time-marking signal are each* 0·1 *msec in duration.* (c) *The end of a similar pulse expanded to show the continuous fall in frequency. Each horizontal bar represents* 0·1 *msec. All traces read from left to right.*

over 100 per second when an object is being investigated. The most characteristic feature is that the frequency of the sound falls steadily by nearly an octave throughout each pulse (figure 2*c*). The frequency at the start of each pulse varies, with the species, between 30 and 120 kc/s; shorter, 'close-range' pulses begin at lower frequencies than the longer ones.

In quiet conditions it is sometimes possible to hear a series of faint clicks as a Vespertilionid bat flies about. Each click occurs at the beginning of a pulse, and by listening to these sounds Dijkgraaf was able to determine the rate of pulse production during various manœuvres [26]. At the higher repetition rates, the clicks follow each other so rapidly that they form a distinct buzzing noise whose pitch is easily determined. Each click consists of a few waves of sound at about 10 kc/s, and its intensity is very much lower than that of the subsequent high-frequency sound. Also it is more pronounced in sick, sleepy, or very young bats, and less in those that show the greatest skill in avoiding obstacles. This component therefore appears to play little part in echolocation, but it may be a useful guide to the way the larynx makes the pulses. If the cricothyroid muscles are paralysed by cutting their motor nerves, the bat produces pulses whose frequency remains constant at about 10 kc/s [15]. This may represent the frequency of the chords at their resting tension and subsequent changes in frequency may be in some way produced by muscular action.

The pulses of Rhinolophids are much more constant in form [25]. They last for 40–100 msec and are of very constant frequency, between 85 and 100 kc/s according to the species, although a fall in frequency may be observed during the last few milliseconds (figure 1). Pulse production is co-ordinated with breathing, which is itself synchronized with wing movements in the flying animal. The

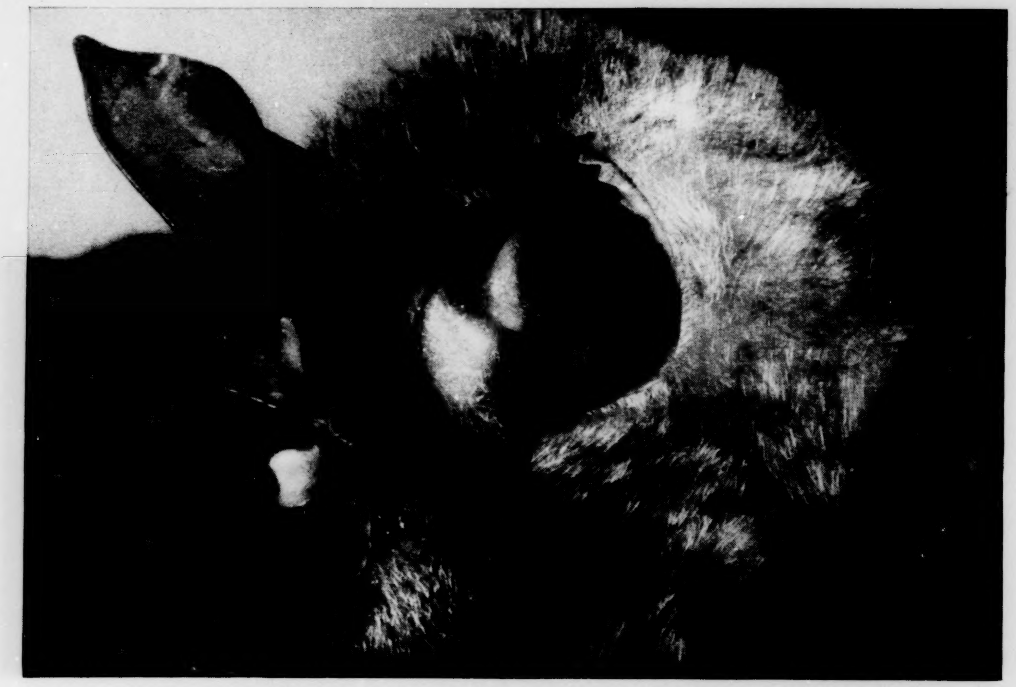


FIGURE 3 – *The head of* Rhinolophus ferrum-equinum (*Rhinolophidae*), *the greater horseshoe bat* (×3). *The nostrils are set close together at the centre of the complex nose-leaf that gives this animal its name. The antitragus is here seen edge-on across the base of the ear.*

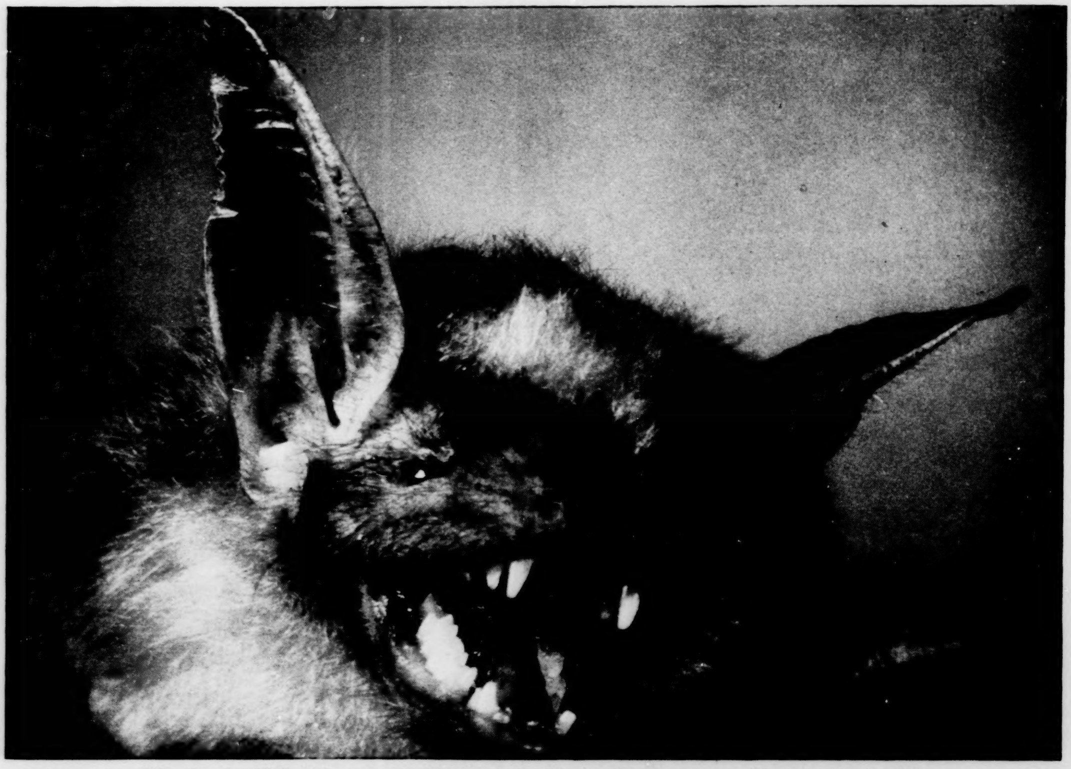


FIGURE 4 – *The head of* Myotis myotis (*Vespertilionidae*), *the mouse-eared bat* (×2·7). *The ears are long but are relatively much smaller than in* Plecotus (*figure* 5).

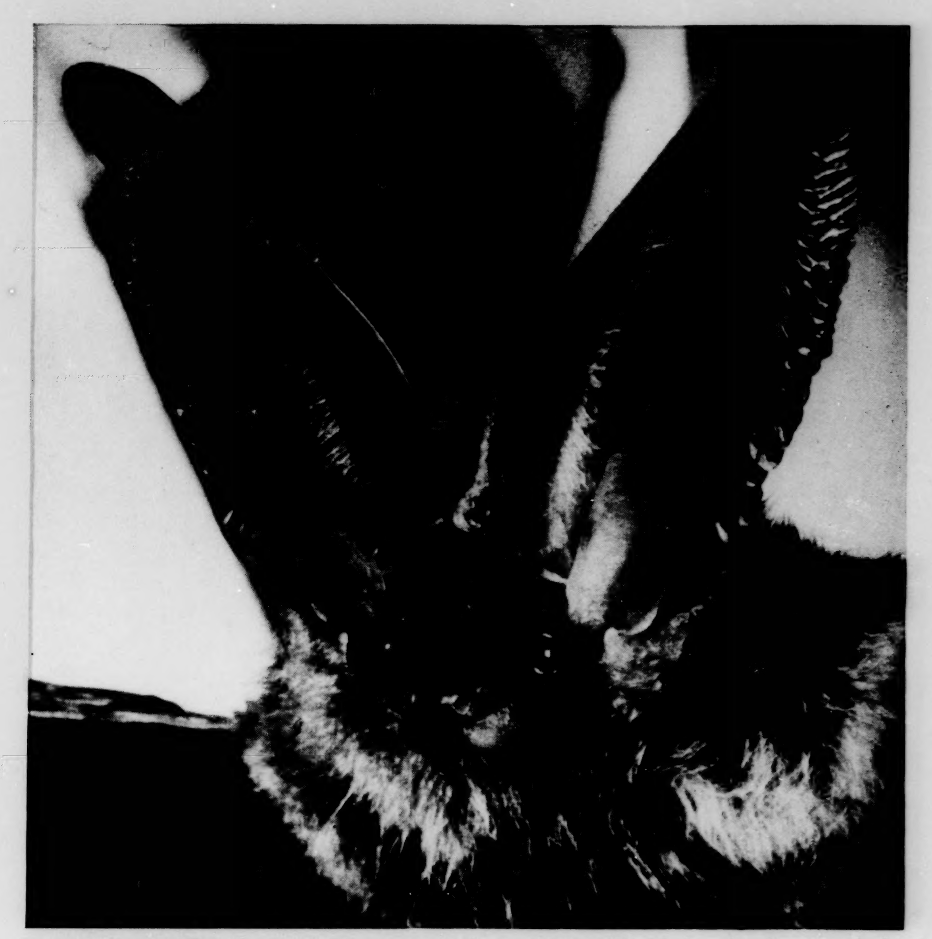


FIGURE 5 – *The head of* Plecotus auritus (*Vespertilionidae*), *the long-eared bat* (×3). *The external ears are enormously developed and are held forwards in flight. The tragus is long and pointed, and the snout has no appendages.*

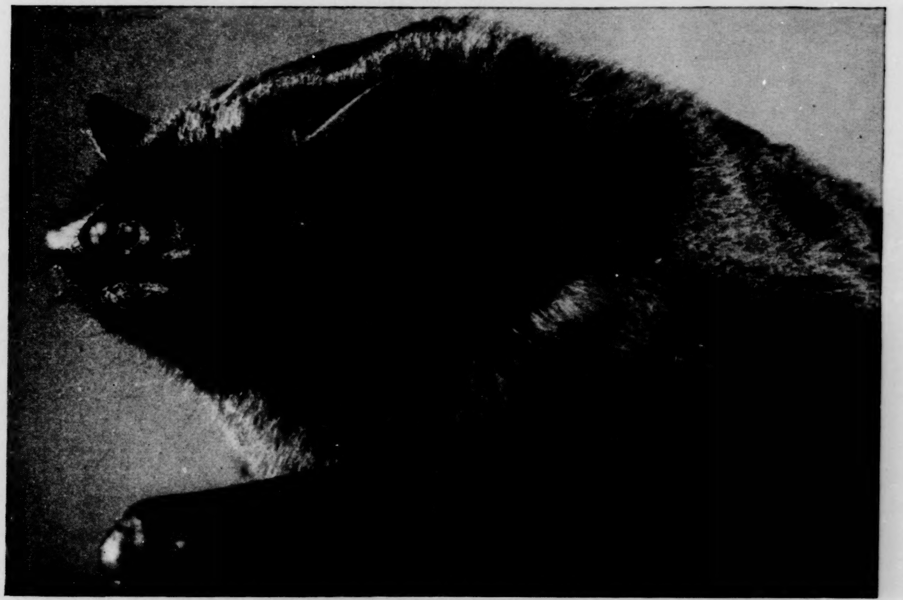


FIGURE 6 – *The head of* Nyctalus noctula (*Vespertilionidae*), *the noctule* (×2·5). *The ears, here seen in a relaxed position, are rounded, with an almost reniform tragus.*

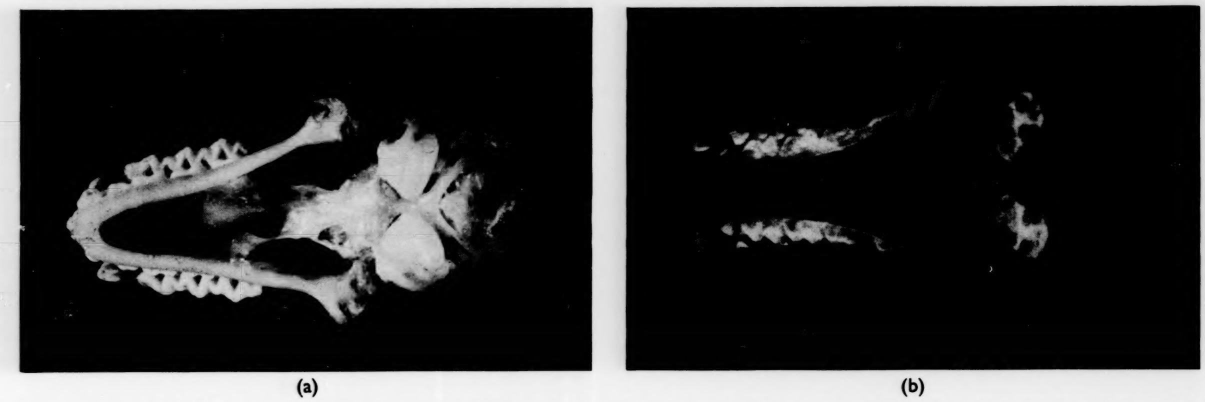


FIGURE 7 – (a) *Ventral view of the skull of* Rhinolophus ferrum-equinum (×2·6). *The large proportions of the cochleae are apparent, but the bullae which enclose the middle ear are restricted to rings of thin bone on the outer side of each cochlea.* (b) *An X-ray photograph of the same skull to show the full extent of the cochleae.*

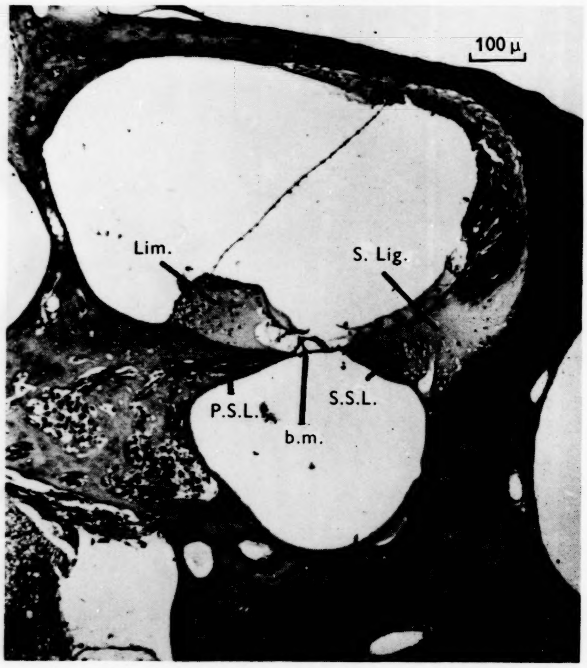


FIGURE 8 – *A photomicrograph of a section of one turn of the cochlea of* Rhinolophus ferrum-equinum. *b.m. = basilar membrane; Lim. = limbus; P.S.L. = primary spiral lamina; S. Lig. = spiral ligament; S.S.L. = secondary spiral lamina.*

pulse-repetition rate therefore seldom exceeds five or six per second in flight, and is not so variable with behaviour as in the Vespertilionids. The pulses of both these families show a very low harmonic content, although other bats may produce marked second and third harmonic components [20].

The ears of bats are highly modified for the reception of high-frequency signals. The external ear is always large—its length may exceed that of the body (figure 5)—but its structure varies considerably. The Vespertilionidae have a fairly simple immovable lobe, with a well-developed tragus looking like a second, smaller ear in front of it. Several suggestions have been made concerning the function of this 'earlet', but so far none has been substantiated. The shape of the tragus usually resembles that of the pinna (figures 4–6), which suggests that it plays some part in determining the acoustic properties of the ear.

The Rhinolophidae have no tragus, but another lobe, the antitragus, is developed as a fold in front of the pinna and forms a funnel around the ear canal (figure 3). The two lobes do not meet at their bases on the inner side: there is thus a second opening to the meatus which faces forward and downward along the snout. The ears of these bats are freely and independently mobile and, when the animal is awake, are kept in constant motion by complex musculature on the head. They appear thus to be actively searching for the sources of sounds or echoes, whereas the Vespertilionidae can turn only the whole head. Möhres [25] has shown that, in addition to these searching movements, the ears are also vibrated forwards and backwards at rates up to 50 times per second, the tip of the ear often moving through an arc of 8–10 mm. This unusual action only occurs while the bat is emitting exploratory pulses, and it may be responsible for a low-pitched buzzing sound that is produced at this time. It seems to be an important part of the echolocation mechanism, since, if the ears are rendered immobile by denervating the appropriate muscles, the bat is disorientated in flight. Later the animal learns to compensate by performing rapid movements of the whole head, and the ability to orientate is regained to some extent [28].

The muscles of the middle ear are very well developed. These are the tensor tympani and stapedius, responsible for tensioning the chain of ossicles that conducts sound from the ear-drum to the inner ear. The bulla is composed of very thin bone and is restricted in extent to the external side of the cochlea (figure 7*a*). The cochlea is very large and occupies much of the posterior part of the skull, especially in the Rhinolophidae, where the two receptor organs almost meet in the midline (figure 7, *a* and *b*). The basilar membrane is very narrow and appears to be under tension, since its supporting structures are very well developed (figure 8). On the inside are a thick spiral lamina and a very high limbus; to the outside the spiral ligament is extremely large and is further strengthened by a second spiral lamina of bone. Many features of this receptor are not yet understood, but they represent an extreme form of the pattern usually associated with hearing in the high-frequency ranges [29]. This conclusion is supported by a certain amount of experimental evidence. Cochlear microphonic potentials have been obtained from Vespertilionids as far as 98 kc/s, the limits of the apparatus used [30]. In other experiments, bats were trained to expect food when sounds of various pitch were heard, and responses were obtained up to 200 kc/s [31]. By comparison, hearing in man seldom extends above 17–20 kc/s.

Although the total size of the brain may be extremely small, the auditory regions of the bat's brain are extraordinarily well developed [15]. The visual centres, by contrast, are greatly reduced. Grinnell and Griffin [32] have recorded changes in electrical potential at several levels in response to sound stimuli but especially at the posterior colliculus. They found that the greatest responses are evoked by short pulses of sound and that pairs of pulses can be resolved when separated by as little as 1–5 msec. Inhibitory interaction between signals from the two ears was observed when the sound was projected from certain directions. There was little evidence of noise rejection at this level, but sharply tuned responses were obtained to brief tones as high as 150 kc/s.

These high frequencies are an important feature of echolocation, as they increase the resolution that can be achieved. In general, waves are reflected specularly only by objects whose dimensions are very much greater than one wavelength. As size decreases, the received energy becomes reflected more widely, until objects that are very small compared with the wavelength scatter incident energy equally in all directions. Wavelength is inversely proportional to frequency, and the sounds used by bats, in the range 25–120 kc/s, have wavelengths of 15–3 mm. They are thus able to produce sharp echoes from quite small objects, but the successful detection of targets smaller than this shows that to some extent useful echoes may be obtained from scattered signals.

The range at which detection may occur has been estimated by several methods. Using the increase of pulse-repetition rate of Vespertilionids as a criterion of detection, Griffin [15] has estimated that barriers of wire may be detected indoors at about 2 m, although no avoiding action was taken until the bats were much closer. The same bats in the open will dive 5–6 m to investigate small objects thrown into the air. However Dijkgraaf and Möhres estimated, by a series of training experiments, that accuracy of discrimination extends to a range of only about 50 cm [31, 25]. Rhinolophid bats, on the other hand, appear to perceive their surroundings accurately to a range of about 6 m, although resolution probably decreases with distance.

A further difference between the two families is seen when the ears are temporarily incapacitated. All bats are completely disorientated if the external ears are carefully plugged on both sides, but a Rhinolophid bat is able to fly perfectly well with one ear free, and to avoid obstacles with ease. In strange contrast, the Vespertilionid bat deafened on one side only is loth to fly, and when forced to do so it blunders about as clumsily as when totally deafened.

To summarize, the Vespertilionidae produce many short, frequency-modulated, pulses through a wide angle; their ears are not mobile, and both must be functioning. The Rhinolophidae make fewer, longer, pulses of very constant frequency, and concentrate the sound into a beam with which they scan their surroundings. The ears move independently in a complex manner; orientation may be achieved monaurally; and the bats can probably probe accurately to greater ranges.

How do these mechanisms operate? What information do the bats obtain about nearby objects? Are there two or more distinct methods, or are they variations of a single one? These fundamental questions cannot yet be answered, but several theories have been proposed in attempts to explain the mechanism behind the observed phenomena. The first, that of Hartridge, resembled the principles of early radar in many ways [33]. He supposed that the bat can estimate the range of its targets by measuring the time delay before an emitted sound returns as an echo. Normal binaural location of the direction from which the echo comes will then give precise information about the relative position of the object concerned. The very short pulses of the Vespertilionidae were considered to be suitable for such a mechanism, as they contain sharp peaks of energy that could be used to mark short intervals of time; the form of Rhinolophid pulses was not then known.

Under close examination, several points of difficulty arise regarding this early theory [34]. The time delays involved are very short, and their measurement must be extremely accurate to explain the skill observed. A central mechanism capable of this accuracy must be envisaged. It is known that the time of arrival of a single sound at the two ears can be compared with great accuracy, but the timing of short intervals by the same ear demands a different type of central analyser. Yet it would seem that range is one of the most important pieces of information, and echo delay its most direct means of measurement.

Again, it is difficult to understand how the bat can hear very faint echoes so soon after it has made such a loud noise itself. It is unlikely that much attenuation can be effected within or around the head of such a small animal, and the mammalian ear generally experiences a rise in threshold following a very loud sound. Hartridge suggested that contraction of the middle-ear muscles by the intra-aural reflex may damp the ear during pulse production and then restore sensitivity for echo reception. But the very high speeds of reflex action and muscle relaxation needed make such an idea untenable. Direct motor control of these muscles, synchronized with those of the larynx, might achieve the required result, but there is no evidence or precedent for such an arrangement.

Furthermore, at the very close ranges involved in the interception of insects, the echo front must return before production of the pulse is completed. This overlap would be expected to mask the fainter echo-signal. Griffin has suggested that frequency modulation of the pulses may overcome this difficulty, since the echo delay will ensure that pulse and echo are never heard at the same frequency at the same time, but the difference must be small at very close ranges. The problem could also be reduced if the bat employs the principle of the recently developed chirp-radar [35]. This system uses frequency-modulated pulses similar to those of the Vespertilionidae, and the receiver incorporates a delay network whose effect varies with frequency. If the pulse front is delayed more than subsequent parts, the emergent pulse is compressed in duration, and overlapping echoes may be separated. Is it physiologically possible for the bat's ear to contain such a frequency-sensitive delay network?

The ability to cope with multiple echoes demands that the timing device, whatever its mode of operation, should be able to handle a series of intervals simultaneously and to discriminate between them. The extent to which some bats can deal with such situations would place very great, though not necessarily insuperable, demands on such a system.

Möhres has rejected the idea of delay-time measurement for the Rhinolophids because of the long duration and low repetition-rates of the sounds made by these bats. There are no sharp energy-peaks in these pulses, so that only the relatively infrequent fronts and trailing edges of pulses could be used for timing, and chirp-radar principles cannot apply. Instead, Möhres suggests that the bats observe the loudness of echoes while scanning with their beam of ultrasound and with their presumably highly directional ears. The bearing of echo targets is then easily obtained from proprioceptive information and the range could be obtained by a process of triangulation, although the base-lines involved are very small. This method could operate monaurally and therefore satisfies the observations on this point.

The problem of masking is here even greater than for Vespertilionids. The sound does not change frequency, and the duration of each pulse will ensure that overlap of pulse and echo occurs up to a range of several metres. It is hard to believe that the bat could hear any but the loudest of echoes, and minute observation of their intensities would be very difficult.

A general theory applicable to nearly all Microchiroptera has recently been advanced by the author [34] in an attempt to overcome some of these problems. It is rather striking that the maximum ranges at which each type is thought to locate objects accurately are those at which their respective pulses and echoes just overlap. Possibly the overlap, which would seem to mask at least part of each echo, may be a necessary part of the detection mechanism. It is suggested that under these conditions the bat may not attend to the echo itself; instead, he listens to beats between the echo and the original call. If beats occur at a

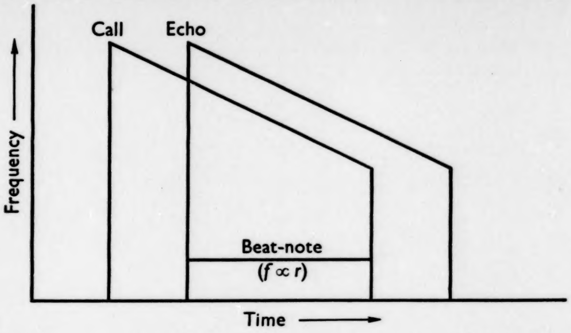


FIGURE 9 – *A diagram of the possible construction of a beat-note from a Vespertilionid pulse and its echo. The beat frequency* (*f*) *is directly proportional to the range* (*r*) *of the target.*

frequency greater than the lower limit of hearing, they could be heard as a separate note, a beat-note.

For the frequency-modulated pulses of the Vespertilionids, the frequency of the beat-note will be characteristic of the echo delay and therefore of the range of the target (figure 9). Frequency analysis by the cochlea could thus provide an accurate method of measuring the very short time-intervals involved. Since the frequency of the pulse seldom changes by more than an octave (a factor of two), the beat frequencies will nearly always be below those of the pulse and echo, so that problems of masking will be much reduced. Multiple targets may be distinguished, as each echo produces a separate beat-note and the cochlea is known to be able to discriminate between simultaneous notes of different frequencies. A comparison of beat-notes produced in each ear could also give directional information.

The same idea has been put forward independently by L. Kay [36]. He further suggests that an extension of range could be achieved if the bat repeats each pulse to itself after a controlled delay and so produces beats with later echoes. Without a provision of this kind, one must assume that the bats that detect wires at distances greater than half the pulse-length in air must be using another mechanism. But detection of an object does not necessarily indicate that the bat possesses exact knowledge of its position. Possibly little information is gained from the discrete echoes heard at first, and accuracy is obtained only when the range is reduced and overlap occurs. The agility of bats, which can turn in 30 cm or less, makes it very difficult to judge how much information they have when 2 m from an obstacle. Nevertheless, the bats' initial directional perception of insects appears to be very good [15].

The beat theory applied to Rhinolophid pulses provides a sensitive method for the detection of Doppler shifts (figure 10). The bat flying towards an obstacle could measure the approach velocity, as this is proportional to the beat frequency. But, probably more important, it provides a method of preventing masking. At the relatively high speed of 10 m/s, a pulse of 100·00 kc/s (as used by *Rhinolophus hipposideros*) will return as an echo of 106·25 kc/s and so produce a beat-note of 6·25 kc/s. This is probably the maximum value that will be encountered, and the original pulse will hardly interfere with its detection. At the same time the mechanism is extremely sensitive to the very low relative velocities. Location of the target may then be achieved by observing the loudness, not of the echo as Möhres suggests, but of beat-notes from it and the call.

For this method to work, the ears of the Rhinolophid must be highly directional, and here the very rapid vibration of the pinnae may help. If each pinna acts as a reflector for collecting echoes, its own movement will introduce a range of Doppler shifts which will, at any instant, be proportional to the velocity of each part of the collecting surface. Thus the ear vibrating at 50 c/s will produce a range of beat-notes (figure 11). The 100 c/s component of this mixture will predominate, as it will synchronize with the ear movements. The effect will be greatest for an echo returning in the direction of ear movement and will decrease to either side. Even the stationary bat can detect stationary objects by this means, as the velocity component is supplied artificially. Of course, if the original pulse from the nostrils were heard after reflection from the moving pinna, it would experience Doppler shifts similar to those of the echo and no particular

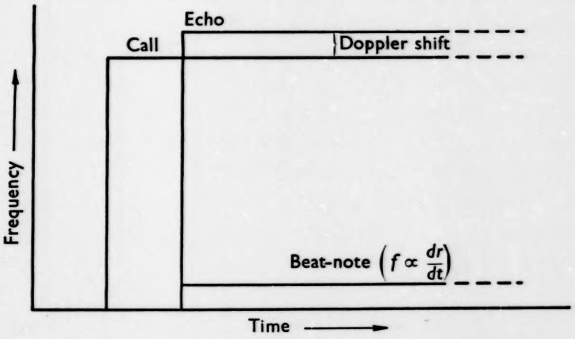


FIGURE 10 – *A diagram showing the production of a beat-note from a Rhinolophid pulse. The beat frequency is proportional to the rate of change of range* (*dr/dt*), *that is, to the relative velocity of bat and target.*

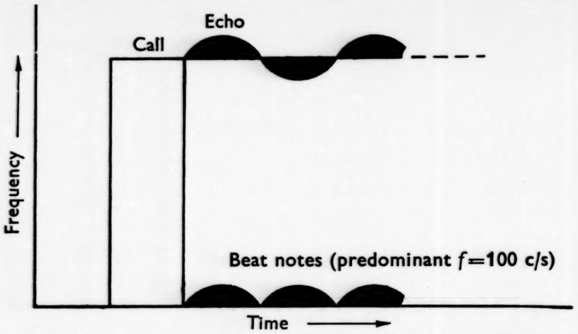


FIGURE 11 – *A diagram of the beat-notes that could be produced by ear vibration in a Rhinolophid. The component whose frequency is twice that of the ear movements (or higher even multiples) will remain coherent and so will predominate. The beat-frequency scale and Doppler-shift scale are here exaggerated for clarity.*

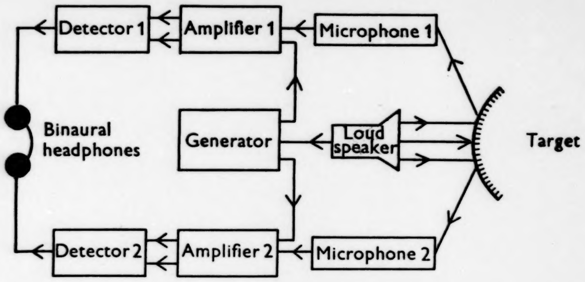


FIGURE 12 – *A block diagram of the apparatus used to test the ability of the human ear to detect objects by beat-note echolocation. The generator produces different types of bat-like sounds, and beat-notes produced with the echoes are heard through headphones. The 'vibrating ear' reflectors for each microphone have been omitted from the diagram for simplicity.*

beat-note component would predominate. It is therefore suggested that the second opening to the ear canal, at the non-moving base of the pinna, may act as a pathway for the 'direct' signal. As may be seen in figure 3, the ventral aperture faces along the snout towards a deep groove in the periphery of the nose-leaf 'transmitter'.

One of the methods of detecting beat-notes is to add the two original signals together and then to subject them to non-linear distortion. The first action occurs within the meatus, and there is evidence that the second may occur within the ear. Studies of the cochlear microphonic potentials of cats and guinea-pigs have shown [37] that strongly non-linear characteristics appear when the ear is overloaded at intensities of 1–10 dynes/cm^2. In view of the very much higher intensities produced by bats, it would be surprising if stray sounds from the mouth or nostrils did not reach these levels at the ear. Instead of this signal being an embarrassment by masking echoes, it may be an essential part of the mechanism. The site of distortion within the ear is the source of some controversy, but there is some evidence that the type of distortion best suited to beat-note detection is caused by contraction of the middle-ear muscles [38]. This may explain their well-developed condition in the bat.

Normally beat-notes are not very loud to the human ear, as the degree of distortion present is low, but under optimum conditions, that may well occur in the bat's ear, the beat-note amplitude can approach that of the smaller signal, in this case the echo. In order to see how well the human ear can discriminate the position of objects, a model has been built as shown in figure 12. Distortion is provided by a detector in conjunction with artificially generated bat-like sounds, and the operator is easily able to detect objects in any of the three modes proposed above. Another model using the same principle, but differing in detail, has been constructed by Kay, and similar results have been obtained with it.

The beat-note theory supports rather than invalidates the conclusion already reached by other workers. It attempts only to suggest a method whereby some of the expected results may be obtained and many of the drawbacks avoided. By proposing a common physiological basis for the different systems observed it allows a wide range of intermediate conditions and facilitates speculation about the evolution of these systems. It throws no light, however, on the signal-to-noise problem.

Whether the bats do in fact use one of the many methods suggested so far, or whether they have other more subtle means at their disposal, can only be decided by further investigation. This promises to be very exciting, and a better understanding of the bat's speed, accuracy, and resistance to jamming may well be of interest in fields far removed from the study of small mammals.

REFERENCES

[1] LISSMAN, H. W. *J. exp. Biol.*, **35**, 156, 1958.
[2] LISSMAN, H. W. and MACHIN, K. E. *J. exp. Biol.*, **35**, 451, 1958.
[3] GRIFFIN, D. R. *Proc. nat. Acad. Sci., Wash.*, **39**, 884, 1953.
[4] NOVICK, A. *Biol. Bull., Wood's Hole*, **117**, 497, 1959.
[5] MEDWAY, LORD, *Nature, Lond.*, **184**, 1352, 1959.
[6] NOVICK, A. *J. exp. Zool.*, **137**, 443, 1958.

[7] Griffin, D. R., Novick, A., and Kornfield, M. *Biol. Bull., Wood's Hole*, **115**, 107, 1958.
[8] Möhres, F. P. and Kulzer, E. *Z. vergl. Physiol.*, **38**, 1, 1956.
[9] Kulzer, E. *Ibid.*, **43**, 231, 1960.
[10] Schevill, W. E. and Lawrence, B. *Breviora, Mus. comp. Zool. Harvard*, **53**, 1, 1956.
[11] Kellogg, W. N. *J. acoust. Soc. Amer.*, **31**, 1, 1959.
[12] Dijkgraaf, S. *Isis*, **51**, 9, 1960.
[13] Hartridge, H. *J. Physiol.*, **54**, 54, 1920.
[14] Pierce, G. W. and Griffin, D. R. *J. Mammal.*, **19**, 454, 1938.
[15] Griffin, D. R. 'Listening in the Dark.' Yale University Press. 1958.
[16] Griffin, D. R., Webster, F. A., and Michael, C. R. *Anim. Behav.*, **8**, 141, 1960.
[17] Webster, F. A. (Unpublished observations.)
[18] Griffin, D. R. and Grinnell, A. D. *Science*, **128**, 145, 1958.
[19] David, E. E. and Kaiser, J. F. Reported in *Wireless World*, **66**, 467, 1960.
[20] Griffin, D. R. and Novick, A. *J. exp. Zool.*, **130**, 251, 1955.
[21] Novick, A. *Ibid.*, **138**, 81, 1958.
[22] Möhres, F. P. and Kulzer, E. *Verh. dtsch. zool. Ges., Erlangen*, **59**, 1955.
[23] Robin, M. H. A. *Ann. Sci. nat.*, **12**, 1, 1881.
[24] Elias, H. *Morph. Jb.*, **37**, 70, 1907.
[25] Möhres, F. P. *Z. vergl. Physiol.*, **34**, 547, 1953.
[26] Dijkgraaf, S. *Experientia*, **2**, 438, 1946.
[27] Griffin, D. R. *Ibid.*, **7**, 448, 1951.
[28] Möhres, F. P. *Symp. Zool. Soc. Lond.*, **3**, 57, 1960.
[29] Reysenbach de Haan, F. W. *Acta oto-laryng. Stockh.*, suppl. 134, 1958.
[30] Galambos, R. *J. acoust. Soc. Amer.*, **14**, 41, 1942.
[31] Dijkgraaf, S. *Acta physiol. pharm. néerl.*, **6**, 675, 1957.
[32] Grinnell, A. D. and Griffin, D. R. *Anat. Rec.*, **134**, 574, 1959.
[33] Hartridge, H. *Nature, Lond.*, **156**, 490, 1945.
[34] Pye, J. D. *J. Laryng.*, **74**, 718, 1960.
[35] Klauder, J. R., Price, A. C., Darlington, S., and Albersheim, W. J. *Bell Syst. tech. J.*, **39**, 745, 1960.
[36] Kay, L. *Anim. Behav.* (In press.)
[37] Wever, E. G. and Lawrence, M. 'Physiological Acoustics'. Princeton University Press. 1954.
[38] Newman, E. B., Stevens, S. S., and Davis, H. *J. acoust. Soc. Amer.*, **9**, 107, 1937.

Book reviews

RELATIVITY

Relativity, the General Theory, *by J. L. Synge. Pp. xv*+505. *North-Holland Publishing Co., Amsterdam.* 1960. £5 10s. *net.*

Books on general relativity differ in their approaches to the geometrization of gravitation that is made possible by the principle of equivalence, that is, by the curious fact that all bodies fall equally fast in a gravitational field. Some books emphasize the gravitational aspect and make the geometry subsidiary: most take a middle way in which geometry and physics are balanced. I do not think there has ever been such a completely geometrical exposition as the one given by Professor Synge. It is true that the important physical tests are referred to (partly in footnotes), and there is a short reference to the recent check of the theory by the Mössbauer effect; but the major portion of the book and its whole spirit are geometrical.

The exposition employs a new method—it is based on Ruse's function, which is essentially the square of the distance between two events in space-time. This is a sign that we are beginning to be able to describe relativity in terms of concepts that bear some resemblance to those involved in classical physics, where it is described as action at a distance.

The book contains accounts of much recent work has so far been available only in the original papers. There is also a bibliography of over 60 pages, listing work published from the birth of the theory until the present time.

C. W. KILMISTER

ELEMENTARY-PARTICLE PHYSICS

Progress in Elementary Particle and Cosmic Ray Physics, *Vol. V, edited by J. G. Wilson and S. A. Wouthuysen. Pp. xii*+461. *North-Holland Publishing Co., Amsterdam.* 1960. 85*s. net.*

In 1952, when the first volume of the series 'Progress in Cosmic Ray Physics' appeared, the chief sources of most elementary particles were the high-energy fluxes from cosmic radiation. In the preface to the third volume (1956) it was noted that the operation of the great accelerating machines had brought a new and dominant approach to much of the experimental work on strange particles, and it had to be decided whether elementary-particle physics—as distinct from pure cosmic-ray physics—should be excluded from subsequent volumes. The editors concluded that this was not desirable, and the two fields of interest are now combined in the new title of the fifth volume of the series, which contains reviews on weak interactions by A. Lundby; phenomenology of nucleon-nucleon interaction by J. Gammel and R. Thaler; theory of anti-nucleons by J. McConnel; observations on cosmic ray jet-interactions in nuclear emulsions by D. H. Perkins; and absorption and decay of negative muons by R. M. Tennant.

Admirable as all the articles are, I personally found the first two contributions particularly rewarding reading. These two articles occupy nearly half the volume, and both are timely, as certain aspects of the subjects treated are currently coming to a definitive close. The determination of a unique set of proton-proton-scattering phase shifts up to energies around 300 MeV appears nearly complete, representing the end of a quest that began some twenty-five years back, and it is proper that a review should appear from two authors who have been among the most active in this quest. Similarly, β-decay of the neutron and the muon seems well understood, and although a number of review articles on weak interactions have been published in recent years, these have been written mostly by theoretical physicists. Lundby's, coming from a distinguished experimental physicist, supplies the right emphasis in regard to the 'experimental nuances' of the subject and is altogether welcome.

This is perhaps the place to mention that I still think there is need for a third type of review article on weak interactions—an article tracing the history of the complex interrelation of experiment and theory, telling the tangled story of how false theories led to, dare one say, false experimental findings in the last few years, and *vice versa*. Grodzins (*Proc. Nat. Acad. Sci.*, **45**, 399, 1959) and Blackett (Rutherford Memorial Lecture, 1958) have made a start in this direction but there is still need for a longer review, which would inevitably be charged with many moral lessons. Ideally it should be written by someone who is at once a great theorist as well as a great experimental physicist. There just are not enough writings of this sort in scientific literature.

A. SALAM

MATHEMATICAL PHYSICS

The Theory of Brillouin Zones and Electronic Structure in Crystals, *by H. Jones. Pp.* 268. *North-Holland Publishing Co., Amsterdam.* 1960. 60*s. net.*

It is most important, in theoretical physics, to distinguish between exact and approximate mathematical results. The problem of calculating the energy of an electron moving in the periodic field of a crystal lattice has been central to the theory of solids for the past thirty years, and has still not been completely solved. But there are a great many perfectly precise statements one can make without ever solving the equations of motion, from a study of the symmetry properties of the crystal structure. Some elegant theorems can be established from the geometry of the lattice and the formal algebra of group theory. Before one embarks upon a numerical computation—and, again, when one has reached the other shore—one must check against these theorems and make sure they are fully satisfied. Professor Jones has made this task much easier by setting out the whole theory in simple practical terms, in simple practical prose, complete with 66 tables, 94 figures, and indeed almost all the detailed results that one normally needs to know. To the professional mathematical physicist, this book is a valuable instrument, as much so as any spectroscope or bubble chamber.

J. M. ZIMAN

PHYSICAL CHEMISTRY

Theory of Unimolecular Reactions, *by N. B. Slater. Pp. ix*+230. *Methuen & Co. Ltd, London.* 1959. 36*s. net.*

This is the first book to be devoted entirely to unimolecular reactions and it gives a full account of the very valuable contributions that the author has made to this subject during the last twenty years. In his treatment, a polyatomic molecule is regarded as made up of a number of classical harmonic oscillators, the motions of which are described in terms of a series of internal co-ordinates, one or more of which must attain a certain critical value for reaction to take place. With the aid of this model—provided that the necessary detailed spectroscopic study and vibrational analysis have been made of the molecules concerned—the author is able to predict, approximately, the frequency factors of unimolecular reactions and, somewhat more successfully, the variation of the velocity constant with reactant concentration. In addition to describing in some detail what is probably the most satisfactory and realistic treatment of the subject yet devised, the author discusses fairly and sensibly the relationship to previous work of his own approach, which he regards not as supplanting but as supplementing and reconciling the older viewpoints.

This is a highly specialized work, and by no means all chemists will want to read, let alone possess, this book. However, all those who are interested in the mechanisms of chemical reactions, and who possess the necessary patience and mathematical background to follow the author's detailed arguments, will find here a valuable and thought-provoking account of an approach to kinetic problems that is of great fundamental importance.

C. F. CULLIS

FREE RADICALS

Stabilization of Free Radicals at Low Temperatures. Summary of the NBS Program, *edited by A. M. Bass and H. P. Broida. Pp. iii*+110. *National Bureau of Standards Monograph* 12. 1960. $1.50.

A research group to carry out work for three years on trapped free radicals was set up in the National Bureau of Standards on September 1956. This group, which was under the direction of H.P. Broida, and was generously supported, provides a unique example of a research team. It was housed in the Bureau, but many of the workers came on temporary leave from industry, and several were from outside the United States. It was engaged in work in a field which it was initially thought might provide a new, powerful, rocket fuel, and yet there was no secrecy. Those who came into the group were free, to a very considerable extent, to pursue their own ideas. This book provides, in eight articles, a good ninety-page account of the work done in the three years. There is also a summary of the symposium that marked the conclusion of the programme. It is true that the work showed that trapping atoms and radicals was most unlikely to provide a rocket fuel. However, it did add greatly to our scientific knowledge in many fascinating and provoking ways. Those who are interested in what such a group could and did achieve will find this book valuable. The articles are most informative, and there is a complete list at the end of the book of the papers published by this group.

J. W. LINNETT

GRAPHITE

Graphite and its Crystal Compounds, *by A. R. Ubbelohde and F. A. Lewis. Pp. xii*+217. *Clarendon Press, Oxford; Oxford University Press, London.* 1960. 35*s. net.*

There is great technical and scientific interest in graphite. The technical interest arises from its extremely high melting point, good conductivity, and attractive nuclear properties; the scientific from graphite's place as the limit of the various aromatic structures and from its being a layered macromolecule.

A great deal of work has been carried out on graphite but much has been wrapped in secrecy, because of manufacturers' reluctance to reveal production techniques and because of the importance of graphite in atomic-energy projects. This book is most welcome, as it surveys the literature of the past century, with over eleven hundred references to work on the crystallography, chemistry, and physics of graphite.

The chemistry of graphite is that of a crystal containing structural defects. The term 'crystal compound' emphasizes that this is a different sort of chemistry from the classical, since, in many of its reactions, the graphite lamellar skeleton is preserved throughout. It is clear that intensive study of the various crystallographic models of graphite and of the structural defects in these, dealt with in the opening chapters of the book, will lead to a better understanding of the electric and magnetic properties, and chemical reactions, so well surveyed in the later chapters. **A. KELLY**

INORGANIC CHEMISTRY

Nouveau traité de chimie minérale, *Vol. XV*, Uranium et transuraniens, *Part I*, Uranium, *edited by P. Pascal. Pp. l+734. Masson et Cie, Paris.* 1960. 115 *NFcs.*

Volume xv, part I, of this treatise deals exclusively with uranium; the forthcoming part II, will be devoted to the transuranium elements. The emphasis in the volume under review is preponderantly on those subjects that are pertinent to nuclear technology: uranium mineralogy, separation of the element from its ores, preparation of the metal and alloys, analysis of uranium-containing materials, and so on. The general chemistry of uranium in solution is covered only briefly. The treatment of major subjects is comprehensive, well organized, and reasonably up to date; most bibliographies extend well into 1958. Figures are numerous, clear, and well chosen. The sections dealing with the alloy systems of uranium and with the physical and mechanical properties of the metal are especially comprehensive, and a chapter devoted to the aqueous corrosion of uranium and its alloys is of considerable technological interest.

Although exception may be taken to some of the more fundamental points of view relating to uranium chemistry—for example, classification of uranium as a member of group VIb of the periodic system—there is no question but that, on the whole, the sub-editors of this volume, R. Caillat and J. Elston, and Professor P. Pascal, the editor-in-chief of this monumental chemical survey, are to be congratulated for their efforts in producing an extremely valuable reference work in the field of modern inorganic chemistry.

G. T. SEABORG

HETEROCYCLIC CHEMISTRY

An Introduction to the Chemistry of Heterocyclic Compounds, *by R. M. Acheson. Pp. xiv+342. Interscience Publishers Inc., New York; Interscience Publishers Ltd, London.* 1960. 35*s. net.*

This admirable book, written by a university teacher and active research worker, gives a clear, concise, and comprehensive account of the main heterocyclic types, including not only relatively well known compounds such as pyrrole, furan, indole, pyridine, and pyrimidine, but also less familar compounds such as azirine, aziridine, azetidine, and quinolizine. Each chapter presents the facts in a logical manner, describing the important physical and chemical properties of representative compounds and, when appropriate, discussing their theoretical significance; considerable attention is devoted to derivatives of biological and technical interest. There are numerous references to review articles and relevant papers, and there is a good subject and substance index. The format and formulae are excellent, and typographical errors are few and for the most part unimportant. The book is most readable and can be strongly recommended to both students and research workers.

N. CAMPBELL

Pyridine and its Derivatives, *edited by E. Klingsberg, Part I, by R. A. Barnes, F. Brody, and P. R. Ruby. Pp. x+613. Interscience Publishers Inc., New York; Interscience Publishers Ltd, London.* 1960. *£*18 7*s. net.*

This is a part of the fourteenth volume in the valuable series of monographs appearing under the general title 'The Chemistry of Heterocyclic Compounds'. It contains two chapters, written by different authors and very different in content. The second chapter has two parts; one gives a very detailed account of the occurrence of the pyridine ring in naturally occurring compounds and the methods by which these derivatives have been separated, the other an equally detailed account of the methods by which the pyridine-ring system has been synthesized in the laboratory. Much of the material is tabulated. This chapter is for experts, and it will be used as a reference section; the material is clearly set out and easily followed.

The first chapter occupies only 97 pages and is very different in character. It succeeds in conveying a lucid yet comprehensive description of the chemistry of pyridine derivatives. This is no catalogue of chemical reactions. Physical and chemical properties are described and, as far as possible, explained in the light of modern theory. This chapter is extremely readable. It is of interest both to the specialist and to the Honours student who requires a short, intelligible account of the chemistry of this typical hetero-aromatic system. Altogether a very useful volume.

P. F. HOLT

ORGANIC CHEMISTRY

The Alkaloids: Chemistry and Physiology, *Vol. VI* (*Supplement to Vols. I and II*) *by R. H. F. Manske. Pp. xii+442.* $14. *Vol. VII* (*Supplement to Vols. II, III, IV, and V*) *by R. H. F. Manske. Pp. xiii+559.* $17. *Academic Press Inc., New York and London.* 1960.

Alkaloid chemistry is one of the traditional fields of endeavour of the organic chemist, and it continues to provide challenging problems of wide interest. The series entitled 'The Alkaloids', edited by the distinguished authority R. H. F. Manske, in part collaboration with H. L. Homes, has provided during the last decade an excellent summary of the field. As the articles are contributed by many authors, the standard attained is not uniform, but on the whole Manske has chosen his authors well. In any case, the series constitutes a unique reference-source for natural-product chemists.

Like other branches of organic chemistry, the subject is no sooner defined than it proceeds to expand at an exponential rate. Supplementary volumes are therefore always urgently needed, and the two books under review serve this purpose for the five volumes already published. It is perhaps unfortunate that definite dates for the literature coverage are not specified, but literature up to about 1957 or 1958 seems to be covered. The present reviewer would suggest that in future volumes all authors should state exactly to what year and month the literature has been thoroughly searched. Even if such dates were embarrassing to the publishers they would be very helpful to the public. Indeed, the publishers might thereby be shamed into more efficient and speedy printing.

The reviewer can recommend these two books as worthy successors to the volumes that have gone before. They will be indispensable not only to libraries, but also to all interested in the chemistry of natural products.

D. H. R. BARTON

GENETICS

Introduction to Quantitative Genetics, *by D. S. Falconer. Pp. ix+365. Oliver and Boyd Ltd, Edinburgh.* 1960. 35*s. net.*

The theory concerning continuous inheritance cannot as yet be used with as much precision in experimental design and in forecasting results as can that concerning discontinuous inheritance, on which it is based. But to the applied geneticist and to many other biologists it is none the less of more interest, for characters of medical and anthropological importance, and plant and animal characters of economic importance, are in general quantitative.

A book, therefore, that not only synthesizes the very diverse and mathematically advanced work of contemporary writers but also avoids their controversies, presents a uniform notation, and uses only elementary mathematics, should be very welcome to research workers in these fields. The latter feature will appeal particularly to the student; no calculus, matrix algebra, or path coefficients appear. An elementary knowledge of diploid genetics, and of statistics (chiefly analysis of variance, correlation, and regression), is all the reader requires.

The first five chapters describe the properties of populations with single-locus segregation; the next five study the concepts arising from extension to multiple loci; these lead to three chapters on selection and three on inbreeding and cross-breeding; and finally there are four chapters on scale, threshold and correlated characters, and natural selection; possibly linkage could have been more extensively treated. The book is meticulous in exposition and well illustrated with experimental material. M. E. WALLACE

PLANT TAXONOMY

Taxonomy of Flowering Plants, *by C. L. Porter. Pp. xii+452. W. H. Freeman & Co., San Francisco and London.* 1960. 44*s. net.*

Plant taxonomy, the first branch of botany, is relatively unfashionable these days, and a good textbook that will interest university students in the subject is sorely needed. There are adequate elementary books and good advanced manuals; none, however, really suitable for university courses. It is particularly disappointing, then, that this book fails to fill the gap. The first few chapters, on principles, nomenclature, methods, literature, and terminology, are quite satisfactory, although experimental methods might have been discussed in more detail—most of the recent advances in taxonomy have been made by their use. The systematic section is the weak point of this book. It is impossible in a text of this type to deal fully with a very large number of families; this is the function of advanced manuals. The result is that no family is dealt with really adequately. A much better plan would have been to deal with selected families in more detail, to give an idea of the range of variation within, as well as between, families. A good deal more material could have been included had the layout of the book not been so extraordinarily wasteful. Fully 15 per cent of the systematic section consists of blank space; some pages have less than one-third of their space occupied. The quality of many of the full and half-page photographs leaves much to be desired; they could have been profitably replaced with text.

This book is one of a series that contains many excellent textbooks. It is difficult to understand why the editors should have passed this one in its present form. It cannot be recommended, although, in the absence of better books, it is usable.

S. R. J. WOODELL

PLANT PHYSIOLOGY

Plant Physiology, *edited by F. C. Steward. Vol. Ia,* Cellular organisation and respiration. *Pp. xxvii+331. Academic Press Inc., New York.* 1960. $13.

This, the first part (though the second to appear) of a treatise extending to six volumes, contains three chapters. The subjects of two of them are indicated by the sub-title, and the third is devoted to a survey of proteins and enzymes. The chapters are all written by authors with well-established reputations in the fields covered. The treatment is as consistent throughout as can reasonably be expected, and as up to date as the hazards of publication allow in a rapidly moving subject. Moreover the aim is not to give a picture of the fleeting moment but to say what plant physiology is about, and to put it into a reasonable context of morphology and other associated sciences. The aim is also to give a reasoned analysis of the various branches of the subject rather than an exhaustive compilation, or an overall picture. It is, however, claimed that the treatment is sufficiently detailed to benefit the research of specialists and comprehensive enough for the use of students. It would perhaps be rather unkind to consider in detail how far the published chapters achieve two such disparate ideals: they naturally pursue a middle course. In a book intended at least in part for those in training it is unfortunate that the phraseology is rather often slipshod and ambiguous, and that misprints liable to mislead the uninitiated have not been wholly eliminated. Nevertheless, enough has now appeared to make it clear that the treatise as a whole is likely to perform a valuable service in botanical education.

W. O. JAMES

PHOTOPERIODISM

Photoperiodism in Plants and Animals. Proceedings of the Conference on Photoperiodism, October–November 1957, *edited by R. B. Withrow. Pp.* 922. *American Association for the Advancement of Science, Washington; Bailey Bros. and Swinfen Ltd, London.* 1959. £6 13*s. net.*

Night and day impose diurnal rhythmicity of photosynthetic and other activities; alternation of the seasons requires devices such as dormancy and migration to ensure survival. These seasonal changes are, in many organisms, effected by photoperiodism (sensitivity to the pattern of exposure to light and darkness). An inborn rhythmicity, with a period of approximately 24 hours, is also a common biological property, and the rhythm phase is usually determined by light.

A symposium to survey the facts and theories of photoperiodism was held in October 1957. Its programme seems to have been devised in the hope that diurnal rhythmicity might account for photoperiodic responses to unnatural light-cycles. No individual is likely to possess all the facts available on, and relevant to, photoperiodism, so that bringing all the data together is a most desirable objective.

From the title and bulk of this book one might expect coverage of the whole field, but this is hardly achieved. It deals first with pigments, then with pathways leading to the various effects; plants first, then animals. It consists mainly of reviews; discussion is replaced by a few contributions of later date. On the animal side, diurnal rhythm is well treated, and so is photoperiodicity of insects and of birds; but plumage changes are omitted. One page, on a species said to be unaffected by day-length, supplies the only detail of photoperiodicity in mammals.

JOHN HAMMOND, JR.

Short notices of books

(These notices are descriptive rather than critical and are designed to give a general indication of the nature and scope of the books.)

A Dictionary of Scientific Terms, *by I. F. Henderson and W. D. Henderson (seventh edition, revised by J. H. Kenneth). Pp. xv+595. Oliver and Boyd, Edinburgh.* 1960. 32*s. net.*

The dictionary is restricted to the fields of biology, botany, zoology, anatomy, cytology, genetics, embryology, and physiology, in which it gives the pronunciation, derivation, and definition of terms. This new edition contains some 15 600 terms, 1750 new ones having been added. Special attention is paid to American usage, and numerous cross-references are included.

Molecular Distillation, *by G. Burrows. Pp. ix+214. Clarendon Press, Oxford; Oxford University Press, London.* 1960. 35*s. net.*

The aim of this book is to bring together the different aspects of the theory and practice of molecular distillation so as to be valuable to the chemist, the physicist, and the engineer. The author considers that his treatment of mixed molecular and laminar gas flow through tubes of arbitrary length is new, and he includes some previously unpublished molecular-distillation composition curves. The special features of gas and vapour flow at low pressures receive detailed treatment.

Zeitschrift für Chemie, *Vol. I, No. I, chief editor G. Horst. Pp.* 32. *Deutscher Verlag für Grundstoffindustrie, Leipzig. DM* 36 *p.a.*

This monthly journal provides reviews on various aspects of chemistry and industry, with a view to making it possible for those working in these fields to keep abreast of progress. This issue includes articles on the mechanism and kinetics of surface catalysis, by S. von Roginski, and on the problem of evolution in terms of the thermodynamics of irreversible processes, by I. Prigogine, as well as a number of short original contributions.

Optical Crystallography (*third edition*), *by E. E. Wallstrom. Pp. x+356. John Wiley & Sons Inc., New York and London.* 1960. 68*s. net.*

This book is an introduction to optical crystallography, and is intended for geologists, mineralogists, chemists, and ceramists. It deals particularly with the theory and practice of the use of the polarizing microscope, avoiding the more difficult and abstruse aspects, and also using drawings to avoid mathematics. The third edition has a completely new text, and has an added chapter on crystal-rotation methods.

Tools of Biological Research, *second series, edited by H. J. B. Atkins. Pp. xii+175. Blackwell Scientific Publications Ltd, Oxford.* 1960. 37*s.* 6*d. net.*

The techniques described in this book, the account of a symposium held at Guy's Hospital in 1959, include electron-spin resonance spectroscopy, paper chromatography, the ultracentrifuge, fluorescent-antibody techniques, phonocardiography, and vectorcardiography. It is mainly directed to surgeons engaged in research, but it contains material that will also interest other research workers in applied biology.

Ultrafiltration, *by L. Ambard and S. Troutmann. Pp. x+67. Charles C. Thomas, Illinois.* 1960. $4.50.

The process of ultrafiltration is interesting because some aspects of it seem analogous to processes that occur at the boundaries of living cells. This monograph deals first with the general principles of ultrafiltration, basing the explanations on a consideration of changes of hydration of the ions involved. The discussion is continued to include the ultrafiltration of plasma and albumin solutions, and the biological implications of these studies.

Introduction to Entomology, *by R. Jeannel. Pp. 344. Hutchinson & Co. Ltd, London.* 1960. 63*s. net.*

The information in this book is arranged under three general headings, anatomy and classification, biology, and paleontology and geographical distribution. The individual chapters cover these fields in some detail, giving particular attention to classification, to the evolution of insects, and to fossil species.

Physiology of Plants, *by P. Font Quer. Pp.* 128. *Arrow Books Ltd, London.* 1960. *Paper back,* 5*s. net; bound,* 10*s.* 6*d. net.*

This is one of the Arrow Science series; it is a translation, from the Spanish, of part II of *Botánica Pintoresca*. It is written for the educated layman and for those starting the study of botany, and it covers such subjects as the functions of water, the chemistry of plants, genetics, and plant movement.

Industrial Electrical Furnaces and Appliances (*second edition*), *by V. Poschkis and John Persson. Pp. xvi+607. Interscience Publishers Inc., New York; Interscience Publishers Ltd, London.* 1960. *£9 net.*

The intention of this book, originally published in two volumes, is to place the practice of electrical heating on a rational basis and eliminate, as far as possible, designing by empirical methods. Heat transfer is studied particularly carefully, as the authors consider that engineers are not always sufficiently appreciative of its relevance to furnace design. The new edition also includes information on oxygen lancing and induction stirring, and on special furnaces, such as consumable-electrode furnaces and skull furnaces.

Hospital Infection: Causes and Prevention, *by R. E. O. Williams, R. Blowers, L. P. Garrod, and R. A. Shooter. Pp. x+307. Lloyd-Luke (Medical Books) Ltd, London.* 1960. 35*s. net.*

This is essentially a practical book, intended for surgeons and sister tutors as well as for bacteriologists and pathologists. The first part of the book deals with the epidemiology of hospital infection, including chapters on staphylococcal infection, gastro-intestinal diseases, and tetanus and gangrene. The second part deals with the control of hospital infection, and considers all aspects, including the design of operating theatres, the use of antibiotics, and the different methods of sterilization.